3M General Offices
3M Center
St. Paul, MN 55144-1000
612 733 1110

AR226-1030a016
MR51622

15 HPP

01 SEP 10 AM 10:13

September 5, 2001

3M 8EHQ-0901-0373

8EHQ-80-373
0000611829T

Document Processing Center (7407)
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460
Attn: TSCA Section 8(e) Coordinator

Dear Section 8(e) Docket Coordinator:

Re: TSCA 8(e) Supplemental Notice on Sulfonate-based Fluorochemicals

With this letter, 3M is providing final reports and other supplemental information related to previous TSCA Section 8(e) notifications. Many of the enclosed items are analytical reports providing blood serum and liver levels of test materials for which the in-life report referring to administered doses has already been submitted to the 8(e) docket. In other cases where the 8(e) notification consisted of preliminary data, we are submitting a final study report.

All of the enclosed items are already in EPA's possession and available in TSCA Docket AR-226. We believe, however, that placing these items in the 8(e) docket may allow for more convenient access to information directly related to previous 8(e) notifications by 3M.

The table below lists the enclosed items and references the study or data which already has been the subject of an 8(e) notification by 3M:

Attached Submission	Related Study/Data Already Filed Under 8(e)
1. Amended Analytical Study, 2(N-Ethylperfluorooctane sulfonamido)-ethanol in Two Generation Rat Reproduction, Determination of the Presence and Concentration of PFOS, M556, PFOSAA, and PFOSA in the Liver and PFOS, M556, PFOSAA, PFOSA and EtFOSE-OH in the Sera of Crl:CDBR VAF/Plus Rats Exposed to EtFOSE-OH, 3M Reference No. T-6316.5, Analytical Report TOX-013, LRN-U2095, June 11, 2001.	Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of N-EtFOSE in Rats, 3M Reference No. T-6316.5, June 30, 1999, full report submitted February 15, 2000 to supplement earlier filing

2001 SEP 25 AM 8:30
RECEIVED
OPPT NCIC

Contain NO CBI

Attached Submission	Related Study/Data Already Filed Under 8(e)
2. Analytical Laboratory Report, Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (CAS Number: 2759-39-3) in the Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage, Laboratory Report No. U2006, Requestor Project No. 3M TOX 6295.9, October 27, 1999. 3. Report Amendment 1, Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats, Argus Research Laboratories, Inc., Protocol 418-008, Sponsor's Study No. 6295.9, April 13, 2000.	Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats, Argus Research Laboratories, Inc., Sponsor's Study No. 6295.9, June 10, 1999, full report submitted February 15, 2000 supplementing earlier filing
4. Analytical Report, Determination of the Presence and Concentration of Perfluorooctanesulfonate, Perfluorooctanesulfonylamide, M556, and M570 in the Liver and Sera Samples, 3M Environmental Laboratory Ref. No. U2636, TOX-028, February 23, 2001	13-Week Dietary Study of N-Methyl Perfluorooctanesulfonamido Ethanol (N-MeFOSE) in Rats, 3M Ref. No. T-6314.1, Covance Study No. 6329-225, dated June 30, 2000, Section 8(e) filing July 24, 2000
* 5. Analytical Laboratory Report, Determination of the Concentration of PFOS, PFOSA, PFOSAA, and EtFOSE-OH in the Sera and Liver of Crl:CDBR VAF/Plus Rats Exposed to N-EtFOSE, 3M Environmental Laboratory Report No. TOX-098, Laboratory Request No. U2402, 3M Ref. No. T-6316.7, February 6, 2001.	Final Report, Oral (Gavage) Developmental Toxicity Study of 2(N-Ethylperfluorooctanesulfonamido)-ethanol in Rats, 3M Reference No. T-6316.7, December 17, 1998, submitted to Section 8(e) docket per letter of August 21, 2000
6. Analytical Laboratory Report on the Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (PFOS) or another metabolite of 2(N-ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE) in Liver and Serum Specimens, 3M Environmental Laboratory Report No. TOX-097, Laboratory Request No. U2452, 3M Ref. No. T-6316.8, February 8, 2001	Final Report, Oral (Stomach Tube) Developmental Toxicity Study of N-EtFOSE in Rabbits, 3M Reference No. T-6316.8, January 11, 1999, submitted to Section 8(e) docket per letter of August 21, 2000
7. Final Report, Alexander, B., Mortality Studies of Workers Employed at the 3M Decatur Facility, University of Minnesota, April 26, 2001.	Preliminary data submitted to Section 8(e) docket in letter of December 15, 2000

Attached Submission	Related Study/Data Already Filed Under 8(e)
8. Final Report, Acute Oral Toxicity Screen with T-3290CoC in Albino Rats, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 0882AR0362, 3M Reference No. T-3290 (40 % K^+PFOSAA in 3 % EtOH, 17 % IPA and 40 % H_20, L-6778, F-6873, Lot 501), November 5, 1982 *[Bibliography entry in Docket AR-226, final report was to be moved to TSCA 8(e) docket]*	Acute Oral Toxicity Screen with T-3290CoC in Albino Rats, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 0882AR0362, 3M Reference No. T-3290 (40 % K^+PFOSAA in 3 % EtOH, 17 % IPA and 40 % H_20, L-6778, F-6873, Lot 501), November 5, 1982, submitted to Section 8(e) docket in August 21, 2000 self-audit letter (which erroneously refers to rabbits rather than rats)
9. Giesy, J.P., and K. Kannan, Accumulation of Perfluorooctanesulfonate and Related Fluorochemicals in Fish Tissue, Michigan State University, June 20, 2001. 10. Giesy, J.P., and K. Kannan, Accumulation of Perfluorooctanesulfonate and Related Fluorochemicals in Mink and River Otters, Michigan State University, June 20, 2001. 11. Giesy, J.P., and K. Kannan, Perfluorooctanesulfonate and Related Fluorochemicals in Oyster, Crassostrea Virginica, From the Gulf of Mexico and Chesapeake Bay, Michigan State University, June 20, 2001. 12. Giesy, J.P. and K. Kannan, Perfluorooctanesulfonate and Related Fluorochemicals in Fish-Eating Water Birds, Michigan State University, June 20, 2001. 13. Giesy, J.P. and K. Kannan, Accumulation of Perfluorooctanesulfonate and Related Fluorochemicals in Marine Mammals, Michigan State University, June 20, 2001.	Preliminary data submitted to Section 8(e) docket May 26, 1999

If you have any questions about this submission, please contact me at (651)737-4795.

Sincerely,

Georjean Adams

Georjean Adams
Manager, 3M Corporate Product Responsibility

Enclosures

MR 51622

Study Title

Oral (Gavage) Developmental Toxicity Study of
2(N-Ethylperfluorooctanesulfonamido)-ethanol in Rats

Analytical Laboratory Report Title

Determination of the Concentration of PFOS, PFOSA, PFOSAA, and EtFOSE-OH in the Sera and Liver of Crl:CD®BR VAF/Plus® Rats Exposed to N-EtFOSE-OH

Data Requirement

Not Applicable

Author

3M Environmental Laboratory

Study Completion Date

At signing

01 SEP 17 PM 3:23

Performing Laboratories

Liver and Serum Analyses

3M Environmental Laboratory
Building 2-3E-09, 935 Bush Avenue
St. Paul, MN 55106

Project Identification

3M Medical Department Study: T-6316.7
Argus In-Life Study: 418-011
Analytical Report: FACT TOX-098
3M Laboratory Request No. U2402

Total Number of Pages

151

EPA-OTS
0008118297

Contains No CBI

8EHQ-80-373

This page has been reserved for specific country requirements.

GLP Compliance Statement

Analytical Laboratory Report Title: Determination of the Concentration of PFOS, PFOSA, PFOSAA, and EtFOSE-OH in the Sera and Liver of Crl:CD®BR VAF/Plus® Rats Exposed to N-EtFOSE-OH

Study Identification Numbers: T-6316.7, FACT TOX-098, LRN-U2402

This study was conducted in compliance with United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations 21 CFR Part 58, with the exceptions in the bulleted list below.

Exceptions to GLP compliance:

- There were two study directors in this study. This study was designed as two separate studies. The in-life phase was considered to end upon weaning of F2 pups and shipment of analytical specimens. The analytical study was considered to start at the receipt of these specimens for analysis. This resulted in having two separate study directors, one for each phase of the same study. However, since the technical performance of each phase was entirely separate, no effect is expected from this exception.
- No expiration date on reagents/solutions labels.
- Sample storage stability was not determined.
- Some analytical reference materials have not been completely characterized.
- QAU did not perform an in-phase inspection during the study.

Marvin T Case — 6 Feb 2001

Marvin T. Case, D.V.M., Ph.D., *Study Director* — Date

John L. Butenhoff — FEB 06, 2001

John L. Butenhoff, Ph.D., *Sponsor Representative* — Date

Kristen Hansen — Feb 01, 2001

Kristen J. Hansen, Ph.D., *Principal Analytical Investigator* — Date

GLP Study—Quality Assurance Statement

Analytical Laboratory Report Title: Determination of the Concentration of PFOS, PFOSA, PFOSAA, and EtFOSE-OH in the Sera and Liver of Crl:CD®BR VAF/Plus® Rats Exposed to N-EtFOSE-OH

Study Identification Numbers: T-6316.7, FACT TOX-098, LRN-U2402

This study has been inspected by the 3M Environmental Laboratory Quality Assurance Unit (QAU) as indicated in the following table. The findings were reported to the study director and laboratory management.

Inspection Dates	Phase	Date Reported to	
		Management	Study Director
11/06/00 – 1/08/00	Data	11/08/00	11/08/00
12/13/00 – 12/15/00	Draft report	12/15/00	12/15/00
12/21/00 – 12/22/00	Draft report	12/22/00	12/22/00

Kevin Ett
QAU Representative

2/2/01
Date

Table of Contents

List of Tables

Study Personnel and Contributors

Study Director
Marvin T. Case, D.V.M., Ph.D.
3M Corporate Toxicology - Medical Department
3M Center, Building 220-2E-02
St. Paul, MN, 55144-1000

Sponsor
John L. Butenhoff, Ph.D.
3M Corporate Toxicology - Medical Department
3M Center, Building 220-2E-02
St. Paul, MN, 55144-1000

Analytical Chemistry Laboratories

Liver and Serum Analyses
3M Environmental Laboratory
Kristen J. Hansen Ph.D., *Principal Analytical Investigator*

3M Lab Contributing Personnel

Lisa A. Clemen
Kelly J. Dorweiler*
Mark E. Ellefson
Sarah A. Heimdal*
Marlene M. Heying*
Harold O. Johnson
Kelly J. Kuehlwein*
Sally A. Linda*
Joseph C. Pilon*
Ian A. Smith*
Kathleen M. Stock*
Bob W. Wynne*
Richard D. Youngblom*

*Contract lab professional service employees

Location of Archives

All original raw data, protocol, and analytical report have been archived at the 3M Environmental Laboratory. The test article and analytical reference standard reserve samples, as well as the specimens pertaining to the analytical phase of this study are archived at the 3M Environmental Laboratory.

Introduction and Purpose

The purpose of the analytical study is to quantify levels of PFOS, PFOSA, PFOSAA, and EtFOSE-OH in sera samples and liver samples collected from rats exposed to N-EtFOSE-OH. This study was initiated on 30 September 1998.

Test System

Nineteen presumed pregnant female rats were assigned to each of five dosage groups (Groups 1 through V). Table 1 outlines the dosage levels and the number of rats per group designated for collection of analytical samples for Argus In-life study 418-011.

The test system species and strain selected was the Crl:CD®BR VAF/Plus® (Sprague-Dawley) rat received from Charles River Laboratories, Inc., permanently identified using a Monel® self-piercing ear tag.

Table 1. Test System Population Demographics and Dosage Levels for Study (418-011)

Dosage Group	Dosage (mg/kg/day)	Number of Rats
I	0	3
II	1	5
III	5	3
IV	10	3
V	20	5

Specimen Collection and Analysis

Sample specimens were collected from Argus (study 418-011) and sent to the 3M Environmental Laboratory for analysis. Liver, sera, fetal, and placental specimens were collected from female rats on day 18 of presumed gestation. Although fetal and placenta specimens were collected, results from these analyses will not be included in this report. A separate report may be issued for fetal tissue data. The number and type of specimens collected for analyses in the analytical phase of this study are presented below.

Specimens Collected from Study Groups I through V:
Serum Specimens—19 specimens
Liver Specimens—19 specimens
Fetuses—19 specimens
Placentas—19 specimens

Blood specimens were centrifuged after collection. Serum was then harvested and immediately frozen on dry ice and maintained frozen at -70°C until shipped to the 3M Environmental Laboratory. Liver specimens collected from each animal were excised, weighed, and a sample section (lateral lobe) was frozen and retained at -70°C until shipped to the 3M Environmental Laboratory. Fetuses and placenta were pooled per litter and retained frozen at-70°C until shipment to the 3M Environmental Laboratory. The specimens were shipped to the 3M Environmental Laboratory frozen and on dry ice.

Sera and liver samples were extracted beginning on 30 September 1998 using an ion pairing reagent and either ethyl acetate or methyl-*tert*-butyl ether (MtBE). Liver samples were homogenized prior to the extraction procedure. Sample extracts were analyzed using high-pressure liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ESMSMS) by multiple reaction monitoring. PFOS, PFOSA, PFOSAA, and EtFOSE-OH levels were quantitated by external calibration. Analytical details are included in this report.

Specimen Receipt and Maintenance

The 3M Environmental Laboratory received serum, liver, fetus, and placenta specimens collected at the end of the *in-life* phase of Argus study 418-011 on 9-15-98 and 9-18-98 from Argus. All specimens were received frozen on dry ice and were immediately transferred to storage at -20°C ±10°C.

Control matrices used in liver and sera analyses performed during TOX-098 were obtained from commercial sources and are presented in Appendix A. Samples analyzed at the 3M Environmental Laboratory will be maintained for a period of 10 years and will be stored at the laboratory at -20°C ±10°C.

Chemical Characterization of the Reference Standards

Chemical characterization information on the analytical reference standards used in this study is presented in the tabular form below.

Table 2. Characterization of the Analytical Reference Standards in Study FACT TOX-098

Reference Standard / Formula	Acronym	Source	Expiration Date	Storage Conditions	Chemical Lot Number	Physical Description	Purity[b]
Potassium Perfluorooctanesulfonate $C_8F_{17}SO_3$-K+	KPFOS[a]	3M	2010	Ambient temperature	193	White crystals	NA[c]
		3M	01/01/2010	Ambient temperature	171	Light colored powder	86.4%
N-Ethyl Perfluorooctanesulfonamido ethyl alcohol $C_8F_{17}SO_2N(C_2H_5)CH_2CH_2OH$	EtFOSE-OH	3M	2010	Ambient temperature	936	Amber waxy solid	88.9%
Perfluorooctanesulfonylamido(ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2COO$-Na	PFOSAA	3M	2010	Ambient temperature	617	Yellow to amber liquid	TBD
Perfluorooctanesulfonylamido(ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2COO$- H		3M	2010	Ambient temperature	NB 112999-99	Tan waxy solid	TBD
Perfluorooctanesulfonylamide $C_8F_{17}SO_2NH_2$	PFOSA	3M	2010	Ambient temperature	L2353	Amber brown waxy solid	TBD
1H, 1H, 2H, 2H-Tetrahydroperfluorooctanesulfonic acid $C_8H_4F_{13}SO_3H$	THPFOS	ICN	2010	Ambient temperature	59909	Brown powder	TBD

[a]PFOS—Perfluorooctane ($C_8F_{17}SO_3$-)
[b]Assumed 100% until Certificate of Analysis is completed.
[c]NA—not applicable. This lot is exhausted and cannot be characterized.
TBD—to be determined

Method Summaries

Following is a brief description of the methods used during this analytical study by the 3M Environmental Laboratory. Detailed descriptions of the methods used in this study are located in Appendix C.

Data collected prior to November 1999 was reworked in 2000 to accommodate improvements in data reduction methods. Both the original and "reworked" data are archived; reworked data is presented in the final results. The improved methods are documented in the form of method modifications.

3M Environmental Laboratory

PREPARATORY METHODS

- **FACT-M-1.0,** "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry".
- **FACT-M-3.1**, "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Compounds from Serum or Other Fluids for Analysis Using HPLC-Electrospray/Mass Spectrometry".

  An ion-pairing reagent was added to the sample and the analyte ion pair was partitioned into ethyl acetate. A portion of the ethyl acetate was transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract was reconstituted in 1.0 mL of methanol, and then filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.
- **ETS-8-4.1,** "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"
- **ETS-8-6.0,** "Extraction of Potassium Perfluorooctane-sulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

  An ion-pairing reagent was added to the sample and the analyte ion pair was partitioned into MtBE. The MtBE extract was transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract was reconstituted in 1.0 mL of methanol, and then filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

ANALYTICAL METHODS

- **FACT-M-2.0,** "Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"
- **FACT-M-4.1,** "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry"
- **ETS-8-5.1,** "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"

- **ETS-8-7.0,** "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

  The analyses were performed by monitoring one or more product ions selected from a single primary ion characteristic of a particular fluorochemical using HPLC/ES/MS/MS. For example, molecular ion 499, selected as the primary ion for PFOS ($C_8F_{17}SO_3$-) analysis, was fragmented to produce ion 99 (FSO_3-). The characteristic ion 99 was monitored in the samples and was evaluated versus one or two 1/X weighted, extracted standard curves.

ANALYTICAL EQUIPMENT

The actual analytical equipment settings used in the present analytical phase of this study varied slightly during actual data collection. The following is representative of the settings used during the analytical phase of this study.

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system
Analytical column: Keystone® Betasil™ C_{18} 2x50 mm (5 µm)
Column temperature: Ambient
Mobile phase components:
  Component A: 2mM ammonium acetate
  Component B: methanol
Flow rate: 300 µL/min
Injection volume: 10 µL
Solvent Gradient: 13.5 minutes

Time (minutes)	*%B*
0.0	40%
8.5	90%
11.0	90%
12.0	40%
13.5	40%

Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro II™ Triple Quadrupole system
Software: Mass Lynx™ 3.1, 3.3, and 3.4
Cone Voltage: 30–60 V
Collision Gas Energy: 25–45 eV
Mode: Electrospray Negative
Source Block Temperature: 150°C ±10°C
Electrode: Z-spray
Analysis Type: Multiple Reaction Monitoring (MRM)

Table 3. Negative Ions Monitored in 3M Laboratory Analyses

Target Analyte	Primary Ion (AMU)	Product Ion (AMU)
PFOS	499.0	80.0, 99.0, 130.0
PFOSA	498.0	78.0
PFOSAA	584.0	83.0, 169.0
EtFOSE-OH	630.0	59.0
THPFOS	427.0	80.0

Data Quality Objectives and Data Integrity

The following data quality objectives (DQOs) were indicated in the method performance section of ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry and ETS-8-7.0, Analysis of Perfluorooctanesulfonate or Other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry:

- **Linearity:** The coefficient of determination (r^2) equal to or greater than 0.980
- **Acceptable Spike Recoveries:** 70–130%

Data Summary, Analyses, and Results

Data quality objectives for the analytical phase of this study outlined in the 3M Environmental Laboratory Methods ETS-8-5.1 and ETS-8-7.0 (see Appendix C) were met with the exceptions noted in this report.

Summary of Quality Control Analyses Results

- **Linearity:** The coefficient of determination (r^2) of the standard curve was ≥0.980.
- **Calibration Standards:** Quantitation of the target analytes was based on linear regression analysis 1/x weighted of an opening extracted curve or two extracted matrix curves bracketing each group of samples. High or low points on the curve may have been deactivated to provide a better linear fit over the curve range most appropriate to the data. Low curve points with peak areas less than two times that of the extraction blanks were deactivated to disqualify a data range that may have been significantly affected by background levels of the analyte. Occasionally, a single mid-range curve point that was an obvious outlier may have been deactivated. Quantitation of each analyte was based on the response of one or more specific product ion(s) using the multiple response-monitoring mode of the instrument (see Appendix C, Analytical Methods).
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve (defined as a standard within ±30% of the theoretical value), and is at least two times the analyte peak area detected in the extraction blanks.

Table 4. Determinations of the LOQ in the Analyses of Sera and Liver Extracts

Analyte	Sera Method LOQ (µg/mL)*	Liver Method LOQ (µg/g)*
PFOS	0.025	0.060
PFOSA	0.005	0.120
PFOSAA	0.025	0.060
EtFOSE-OH	0.010	0.060

* Values are approximate
LOQ—Limit of Quantitation

- **Blanks:** All blanks were below the lower limit of quantitation for the quantitative analysis of compounds of interest. Although the matrix blanks were clean, some liver data for G1–G3 should be considered qualitative, as these samples may have been affected by background levels of the analyte found in the method blanks; specific data points affected are noted in the results table. To simplify analyses that were complicated by endogenous levels of fluorochemicals in unexposed rat sera and liver, rabbit sera and liver were selected as a suitable surrogate matrices.
- **Precision:** Precision was not specifically determined within this study, but has been characterized to be better than ±30% for this method.
- **Matrix Spikes:** Matrix spikes and matrix spike duplicates were extracted with each set of sera and liver samples and analyzed during analytical runs at the 3M Environmental Laboratory. Rat sera and liver from control animals were spiked prior to extraction. All target analytes were spiked at approximately 250 ng/mL or 250 ng/g. Sera matrix spikes for PFOSAA and EtFOSE-OH were within ±30% of the theoretical concentration. One matrix spike for PFOS and one for PFOSA were outside of this range (152% and 149%, respectively). The average spike recovery for PFOS in sera was 137% and for PFOSA it was 126%. Matrix spikes prepared in liver (PFOS, PFOSA, PFOSAA, and EtFOSE-OH) were compliant within ±30% for all analytes.
- **Surrogates:** The surrogate (THPFOS) was added to all samples and standards. THPFOS was not used for quantitation, but was used to monitor for gross instrument failure.

Statement of Data Quality

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data are quantitative to ±40%.

Summary of Sample Results

Some PFOS results (those obtained using lot # 171) have been corrected for purity of the analytical reference material. Uncorrected results are noted in the data tables.

- **Samples from Control Animals:** Low levels of PFOS were detected in the liver of the control animals. These levels were significantly lower than those found in the low dose test animals.

- **Samples from Dosed Animals:** In general, levels of the target analytes present in the sera and liver of the test animals increased with dose group. Detailed sample data tables are presented in Appendices D and E.

Statistical Methods and Calculations

Statistical methods were limited to the calculation of means and standard deviations. See Appendix F for example calculations used to generate the liver and serum sample data in FACT TOX-098.

Statement of Conclusion

Under the conditions of the oral development toxicity of N-EtFOSE, PFOS, PFOSA, PFOSAA, and EtFOSE-OH were observed in the sera and liver of pregnant rats dosed with N-EtFOSE-OH during the in-life phase of the study.

Appendix A: Chemical Characterization and Control Matrices

Table 5. Characterization of Test Article in Study FACT TOX-098

	Test Article
Chemical Name	**N-EtFOSE-OH** 2(N-Ethylperfluorooctanesulfonamido)-ethanol
Source	3M
Expiration Date	5/2000
Storage Conditions	Ambient temperature
Chemical Lot #	FM-3929 (30035, 30037, 30039)
Physical Description	Waxy solid
Purity	97.4%

Table 6. Characterization of the Control Matrices Used for Liver and Sera Analyses in Study FACT TOX-098

Control Matrix	Rat Serum TN-A-2001	Rabbit Serum TN-A-2382	Rabbit Liver TN-A-0809	Rabbit Liver TN-A-0810
Source	Sigma	Sigma	CHW	CHW
Expiration Date	2010	2010	2010	2010
Storage Conditions	-20°C ±10°C	-20°C ±10°C	-20°C ±10°C	-20°C ±10°C
Chemical Lot #	17H9306	118H8418	F00012	F00013
Physical Description	Rat Serum	Rabbit Serum	Rabbit Liver	Rabbit Liver

Appendix B: Protocol, Amendments, and Deviations

3M ENVIRONMENTAL LABORATORY

PROTOCOL - ANALYTICAL STUDY
Oral (Gavage) Developmental Toxicity Study of 2(N-Ethylperfluorooctanesulfonamido)-ethanol in Rats

In-vivo study reference number: Argus 418-011
Study number: FACT-TOX-098
Test substance: 2(N-Ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE-OH)

Name and address of Sponsor:

Marvin Case
3M Toxicology Services
3M Center
Building 220-2E-02
St. Paul, MN 55144

Name and address of testing facility:

3M Environmental Technology and Services
935 Bush Avenue, Building 2-3E-09
St. Paul, MN 55106

Sponsor approval date:
Experimental start date: October 9, 1998
Expected termination date: July 16, 1999

Method numbers and revisions:

FACT-M-1.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

FACT-M-2.0, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

FACT-M-3.1, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry

FACT-M-4.1, Analysis of Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry

Author: Lisa Clemen

Kris Hansen	9/30/98	Marvin T Case	24 Nov 98
Kris Hansen Study Director	Date	Marvin Case Sponsor Representative	Date

FACT-TOX-098, U2402
Argus #418-011
Page 1 of 5

1.0 PURPOSE

The analytical portion of this toxicity study is designed to evaluate the levels of potassium perfluorooctane sulfonate (PFOS), or another metabolite of 2(N-ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE-OH) designated by the study director, in the livers of the test system, or other tissues and fluids as necessary.

The in life portion of this study was conducted at Argus Research Laboratories (Argus 418-011).

2.0 REGULATORY COMPLIANCE

This study will be conducted in compliance with the Food and Drug Administration Good Laboratory Practices regulation as stated in 21 CFR 58. Any exceptions will be noted in the final report.

3.0 TEST MATERIALS

3.1 Test, control, and reference substances and matrices

3.1.1 Analytical reference substance: Potassium perfluorooctanesulfonate (PFOS), lot # 217

3.1.2 Analytical reference substance matrix: Rat liver, serum, and whole blood

3.1.3 Analytical control substance: None

3.1.4 Analytical control substance matrix: Rat liver, serum, and whole blood

3.2 Source of materials

3.2.1 Analytical reference substance: 3M Specialty Chemical Division; traceability information will be included in the final report

3.2.2 Analytical reference substance matrix: Argus Research Laboratories; traceability information will be included in the final report

3.2.3 Analytical control matrix:

3.2.3.1 Rat liver – Argus Research Laboratories; traceability information will be included in the final report; or

Rabbit liver – Covance Laboratories; traceability information will be included in the final report.

3.2.3.2 Rat serum - Sigma Chemical Company; traceability information will be included in the final report.

3.2.3.3 Rat whole blood – 3M Toxicology; traceability information will be included in the final report.

3.3 Number of test and control samples. Liver and serum samples will be received for testing from 16 test and 3 control animals for the toxicokinetic portion of the study. Liver and serum samples for testing will be received from 100 test and 25 control

BACK TO MAIN

animals for the developmental portion of the study. Fetus, placenta, or other samples will be tested at the discretion of the Study Director.

3.4 **Identification of test and control samples:** The samples are identified using the Argus Research Laboratories identifiers, which consist of the Argus project number, the animal number, the group designation, and the draw date.

3.5 **Purity and strength of materials:** Characterization of the purity and identity of the reference material is the responsibility of the Sponsor.

3.6 **Stability of test material:** Characterization of the stability of the test material is the responsibility of the Sponsor.

3.7 **Storage conditions for test materials:** Test materials are stored at room temperature. Samples are stored at -20 ± 10 °C.

3.8 **Disposition of test and/or control substances:** Biological tissues and fluids are retained per GLP regulation.

3.9 **Safety precautions:** Refer to the material safety data sheets of chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

4.0 EXPERIMENTAL - Overview

Tissues from animals dosed as described in Argus Research Laboratories Protocol #418-011 will be received for analysis of fluorochemicals. Mated female rats were dosed on Day 6 of presumed gestation, with administration continuing through Day 17. At Day 18, serum and liver samples, as well as fetus and placenta samples, were taken from rats in the toxicokinetic portion of the study. At Day 20 for the rats remaining in the study, samples of serum and liver were taken, as well as fetus and placenta.

Dosage samples will be provided from Argus Research Laboratories for concentration level confirmation. These samples will not be extracted and analyzed according to GLP regulations. The data collected will be provided to the Sponsor as an attachment to the data package.

At the discretion of the Study Director, a series of analytical tests will be performed on select tissues. Initially, all liver and serum samples will be analyzed, using the methods listed in section 5.0, for PFOS by Electrospray/mass spectrometry (ES/MS). On the basis of findings from these analyses, additional samples may be evaluated. If additional analysis is performed, a protocol amendment will be written to add the matrices and methods to the protocol.

At the discretion of the Study Director, select analysis may be performed by a contract laboratory where competence has been demonstrated, using validated analytical methods. If a contract laboratory is used, this protocol will be amended to include the required information. The methods, data, and contract laboratory will be identified in the data package provided to the Sponsor.

5.0 EXPERIMENTAL - Analytical Methods

5.1 For analysis performed by the 3M Environmental Laboratory, the following methods will be used:

5.1.1 **FACT-M-1.0,** Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

5.1.2 **FACT-M-2.0,** Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

5.1.3 **FACT-M-3.1,** Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry

5.1.4 **FACT-M-4.1,** Analysis of Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry

5.2 If analysis is performed at a contract analytical laboratory, copies of the validated methods will be included in the data packet provided to the Study Director.

6.0 DATA ANALYSIS

6.1 **Data reporting:** For analysis performed by a contract laboratory, the contract laboratory will provide all data to the analytical phase Study Director, and copies of the methods will be attached to the data. The contract laboratory and the data it provides will be identified in the data packet provided by the analytical phase Study Director to the Sponsor.

6.2 **Data transformations and analysis:** Data will be reported as the concentration (weight/weight) of the target analyte per tissue or sample, or of the target analyte per unit of tissue or fluid.

6.3 **Statistical analysis:** Statistics used may include regression analysis of the serum concentrations over time, and standard deviations calculated for the concentrations within each dose group. If necessary, simple statistical tests, such as Student's t test, may be applied to evaluate statistical difference.

7.0 MAINTENANCE OF RAW DATA AND RECORDS

7.1 The following raw data and records will be retained in the study folder in the archives according to AMDT-S-8:

7.1.1 Approved protocol and amendments

7.1.2 Study correspondence

7.1.3 Shipping records

7.1.4 Raw data

7.1.5 Electronic copies of data

7.2 Supporting records to be retained separately from the study folder in the archives according to AMDT-S-8 will include at least the following:

7.2.1 Training records

7.2.2 Calibration records

7.2.3 Instrument maintenance logs

7.2.4 Standard Operating Procedures, Equipment Procedures, and Methods

7.2.5 Appropriate specimens

8.0 REFERENCES

8.1 3M Environmental Laboratory Quality System Chapters 1, 5 and 6

8.2 Other applicable 3M Environmental Laboratory Quality System Standard Operating Procedures

9.0 ATTACHMENTS

9.1 Copies of the following validated 3M Environmental Laboratory methods are attached for information purposes:

9.1.2 **FACT-M-1.0,** Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

9.1.2 **FACT-M-2.0,** Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

9.1.3 **FACT-M-3.1,** Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry

9.1.4 **FACT-M-4.1,** Analysis of Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry

9.2 Argus protocol 418-011

Study Title

Oral (Gavage) Developmental Toxicity Study of 2(N-Ethylperfluorooctanesulfonamido)-ethanol in Rats

PROTOCOL AMENDMENT NO. 1

Amendment Date:
22 February 2000

Performing Laboratory
3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification
FACT TOX-098
ET&SS—U2402
Argus Study: 418-011
3M Medical Department Study: T-6316.7

3M Environmental Laboratory

Protocol LRN-U2402
Amendment Number 1

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:***
   The study director for the present study was identified in the protocol as Kristen J. Hansen, Ph.D.
   AMEND TO READ:
   The role of study director for the present study was reassigned to Marvin T. Case, D.V.M., Ph.D., as of the signing of this amendment.
   REASON:
   The role of study director was reassigned in an effort to ensure compliance with Good Laboratory Practice Standards that outline study personnel requirements (refer to 21 CFR Part 58).

2. ***PROTOCOL READS:***
   The sponsor for the present study was identified as Marvin T. Case, D.V.M., Ph.D.
   AMEND TO READ:
   The role of sponsor for the present study was reassigned to John L. Butenhoff, Ph.D., as of 20 January 2000.
   REASON:
   To ensure that the study director does not also carry the duties of study sponsor, the sponsor role was reassigned. In this manner, personnel responsibilities and workload are more evenly balanced.

3. ***PROTOCOL READS:***
   3.1 Test, control, and reference substances and matrices
      3.1.2 Analytical reference substance matrix: Rat liver, serum, and whole blood
      3.1.4 Analytical control substance matrix: Rat liver, serum, and whole blood
   AMEND TO READ:
   3.1 Test, control, and reference substances and matrices
      3.1.2 Analytical reference substance matrix: Rat liver, serum, pooled fetal tissue(s), and whole blood
      3.1.4 Analytical control substance matrix: Rat liver, serum, pooled, fetal tissue(s), and whole blood
   REASON:
   Analysis of fetal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the original protocol.

3M Environmental Laboratory

Protocol LRN-U2402
Amendment Number 1

4. PROTOCOL READS:

7.1 The following raw data and records will be retained in the study folder in the archives according to AMDT-S-8:

- 7.1.1 Approved protocol and amendments
- 7.1.2 Study correspondence
- 7.1.3 Shipping records
- 7.1.4 Raw data
- 7.1.5 Electronic copies of data

7.2 Supporting records to be retained separately from the study folder in the archives according to AMDT-S-8 will include at least the following:

- 7.2.1 Training records
- 7.2.2 Calibration records
- 7.2.3 Instrument maintenance logs
- 7.2.4 Standard Operating Procedures, Equipment Procedures, and Methods
- 7.2.5 Appropriate specimens

AMEND TO READ:

"The original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, and electronic copies of data will be retained in the archives of the 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained in the archives of the facility performing each analysis."

REASON:

To direct subcontract laboratories in the disposition of the items listed above.

5. ***PROTOCOL READS:***

3.8 Disposition of test and/or control substances: Biological Tissues and fluids are retained per GLP regulation.

AMEND TO READ:

3.8 Specimens will be maintained in the 3M Environmental Laboratory specimen archives. All specimens sent to sub-contract laboratories will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the sub-contract laboratory(s) final report. The specimens will be returned with the following documentation: the signed original chain of custody and records of storage conditions while at the sub-contract facility.

REASON:

To define in detail the appropriate disposition of specimens analyzed at subcontract laboratories.

6. ***PROTOCOL READS:***

Method numbers and revisions:
FACT-M-1.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry.
FACT-M-2.0, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry
FACT-M-3.1, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry
FACT-M-4.1, Analysis of Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry

AMEND TO READ:

Method numbers and revisions:
ETS-8-6.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray Mass Spectrometry"
ETS-8-5.1, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Serum Extracts HPLC-Electrospray Mass Spectrometry"

REASON:

New methodologies were implemented following the approval of the original protocol for FACT Tox-098.

Protocol LRN-U2402
Amendment Number 1

Amendment Approval

, March, 2000

John L. Butenhoff, Ph.D., Sponsor Representative — Date

24-March-2000

Kristen J. Hansen, Ph.D., Outgoing Study Director — Date

1 March 2000

Marvin T. Case, D.V.M., Ph.D., Incoming Study Director — Date

3M Environmental Laboratory

BACK TO MAIN

Study Title

Oral (Gavage) Developmental Toxicity Study of 2(N-Ethylperfluorooctanesulfonamido)-ethanol in Rats

PROTOCOL AMENDMENT NO. 2

Amendment Date:

November 21, 2000

Performing Laboratory

3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification

FACT TOX-098
ET&SS LRN-U2402
Argus Study: 418-011
3M Medical Department Study: T-6316.7

3M Environmental Laboratory

Protocol FACT TOX-098
Amendment No. 2

This amendment modifies the following portion(s) of the protocol:

1. PROTOCOL READS:
There is not a principal analytical investigator assigned for this study.

AMEND TO READ:
The role of principal analytical investigator for the study was assigned to Kristen J. Hansen, Ph.D. as of the signing of this amendment.

REASON:
The role of principal analytical investigator was assigned in an effort to ensure compliance with Good Laboratory Practice Standards that outline study personnel requirements.

3M Environmental Laboratory

Protocol FACT TOX-098
Amendment No. 2

Amendment Approval

John L. Butenhoff — 4 DEC 00

John L. Butenhoff, Ph.D., Sponsor Representative — Date

Marvin T Case — 5 Dec 2000

Marvin T. Case, D.V.M., Ph.D., Study Director — Date

3M Environmental Laboratory

Study Title

Oral (Gavage) Developmental Toxicity Study of 2(N-Ethylperfluorooctanesulfonamido)-ethanol in Rats

PROTOCOL AMENDMENT NO. 3

Amendment Date:

November 21, 2000

Performing Laboratory

3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification

FACT TOX-098
ET&SS LRN-U2402
Argus Study: 418-011
3M Medical Department Study: T-6316.7

3M Environmental Laboratory

Protocol FACT TOX-098
Amendment No. 3

This amendment modifies the following portion(s) of the protocol:

1. ***PROTOCOL READS:***

1.0 Purpose: The analytical portion of this toxicity study is designed to evaluate the levels of potassium perfluorooctane sulfonate (PFOS), or another metabolite of 2(N-ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE-OH) designated by the study director in the livers of the test system, or other tissues and fluids as necessary.

AMEND TO READ:

1.0 Purpose: The analytical portion of this toxicity study is designed to evaluate the levels of potassium perfluorooctanesulfonate (PFOS), N-ethyl perfluorooctanesulfonamido ethyl alcohol (EtFOSE-OH), perfluorooctanesulfonylamido(ethyl)acetate (PFOSAA), and perfluorooctanesulfonylamide (PFOSA) in the livers of the test systems, or other tissues and fluids as necessary. Perfluorooctanesulfonylethylamide (PFOSEA) and M556 will be monitored but not used for GLP purposes and will not be part of this study.

REASON:

Specific target analytes are known.

2. ***PROTOCOL READS:***

3.1.1 Analytical reference substance: Potassium perfluorooctanesulfonate (PFOS), lot #217.

AMEND TO READ:

3.1.1 Analytical reference substances: Potassium perfluorooctanesulfonate (PFOS), N-ethyl perfluorooctanesulfonamido ethyl alcohol (EtFOSE-OH), perfluorooctanesulfonylamido(ethyl)acetate (PFOSAA), and perfluorooctanesulfonylamide (PFOSA).

REASON:

Include the additional reference substances used.

3M Environmental Laboratory

Protocol FACT TOX-098
Amendment No. 3

Amendment Approval

John L. Butenhoff — JAN 10 2001

John L. Butenhoff, Ph.D., Sponsor Representative — Date

Marvin T Case — 10 Jan 2001

Marvin T. Case, D.V.M., Ph.D., Study Director — Date

3M Environmental Laboratory

Record of Deviation

I. Identification

Study / Project No.
TOX0098 (LIMS #U2402)

Deviation type *(Check one)*: ❒ SOP X Method ❒ Equipment Procedure ❒ Protocol ❒ Other:

Document number FACT-M-2.1

Date(s) of occurrence 10/02/98 and 10/06/98

II. Description

Required procedure/process:

Section 11.1.: ....The average of two standard curves will be plotted by linear regression, not forced through zero, ...

Actual procedure/process:

Data was originally analyzed as described by the method. However, after the analysis was complete, it was determined that applying a 1/X weighting to the curve dramatically improved method accuracy at the low end of the curve. The original data sets were reworked utilizing the improved practice.

III. Actions Taken
(such as amendment issued, SOP revision, etc.)

Deviation written. Method revalidated utilizing improved practices. Original and reworked data are included with the raw data. The reworked data is reported in the final results.

Recorded by kh

Date 11/20/00

IV. Impact on Study / Project
(completed by Study Director or Project Lead)

The reworked data has a higher degree of accuracy than the original data. There is no adverse impact on the study.

kh 11/20/00

Authorized by Marvin T Case 20 Nov 2000 / John L. Butenhoff 4 DEC 00

Date

Sponsor: John Butenhoff Study Director: Marv Case

Deviation No. 1
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification

Study / Project No. FACT-TOX-098 Argus 418-011

Deviation Type (Check one): ❒ SOP X Method ❒ Equipment Procedure ❒ Protocol ❒ Other:

Document Number(s): ETS-8-5.1

Date(s) of occurrence: 09/28/99 and 10/02/99

II. Description:

Required Procedure/process:
14.4.1 Matrix spike recoveries must be within +/-30% of the spiked concentration.

Actual Procedure/process:
One matrix spike in sera showed a higher recovery for PFOS (152%, average 137%) And PFOSA (149%, average 126%).

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written. The stated accuracy of these data will be changed in the final report to reflect these recoveries.

Recorded By	Date
Lisa A Clemen	12/14/00

IV. Impact on Study / Project

This deviation will have no adverse affect on these data.

kjh 12/14/00

Authorized By *(Study Director / Project Lead)*	Date
John L. Butenhoff DEC 15 2000 Marvin Case 15 Dec 2000	

Sponsor Representative: John Butenhoff Study Director: Marv Case

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 2
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification

Study / Project No. FACT-TOX-098 Argus 418-011

Deviation Type *(Check one)* ☐ SOP X Method ☐ Equipment Procedure ☐ Protocol ☐ Other:

Document Number(s): ETS-8-7.0	Date(s) of occurrence: Entire study

II. Description:

Required Procedure/process:

Section 13.1.6 describes the calculations that should be used to convert extract concentration to matrix concentration.

Actual Procedure/process:

In order to accommodate purity information, the actual calculation used varied somewhat from that written in section 13.1.6. Two additional factors, salt correction and standard purity, were added. The first accommodates the mass difference between the analytical standard (C8F17SO3K) and the target analyte (C8F17SO3-), while the second addresses the purity of the analytical reference material, determined after the study was completed.

kh 12/20/00

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written.

Recorded By	Date
kh	12/20/00

IV. Impact on Study / Project

The updated calculations accommodate new information and are an improvement. No adverse affect on the study. kh 12/20/00

Authorized By *(Study Director / Project Lead)*	Date
John L. Butenhoff 12/22/00 Marvin T Case 21 Dec 2000	

Sponsor Representative: John Butenhoff Study Director: Marv Case

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 3
(assigned by Study Director or Project Lead at the end of study or project)

Appendix C: Extraction and Analytical Methods

This appendix includes the following methods:

Preparatory Methods

FACT-M-1.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry, (8 pages)

FACT-M-3.1, Extraction of Potassium Perfluorooctane or Other Anionic Fluorochemical Compounds from Serum or Other Fluids for Analysis Using HPLC-Electrospray/Mass Spectrometry, (17 pages)

ETS-8-4.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry, (14 pages)

ETS-8-6.0, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry", (14 pages)

Analytical Methods

FACT-M-2.0, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry, (8 pages)

FACT-M-4.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry, (9 pages)

ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry, (11 pages)

ETS-8-7.0, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry, (12 pages)

3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER ANIONIC FLUOROCHEMICAL SURFACTANTS FROM LIVER FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: FACT-M-1.0

Adoption Date: 5/26/98

Revision Date: N/A

Author: Lisa Clemen

Approved By:

Laboratory Manager	5/26/98 Date
Group Leader	5/26/98 Date
Lisa A Clemen Technical Reviewer	5/27/98 Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the extraction of Potassium Perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

1.3 Matrices: Rabbit, rat, bovine, and monkey livers or other livers as designated in the validation report.

2.0 Summary of Method

2.1 This method describes how to extract potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver using ion pairing reagent and 5.0 mLs of ethyl acetate. An ion pairing reagent is added to each sample and partitioned into ethyl acetate. Four mLs of extract is removed to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL methanol then filtered through a 3 cc plastic syringe attached to a 0.2 µm filter into glass autovials.

3.0 Definitions

3.1 None.

4.0 Warnings and Cautions

4.1 **Health and Safety Warnings:**

4.1.1 Use universal precautions when handling animal livers, they may contain pathogens.

5.0 Interferences

5.1 There are no known interferences at this time.

6.0 Equipment

6.1 The following equipment is used while carrying out this method. Equivalent equipment is acceptable.

6.1.1 Ultra-Turrax T25 Grinder for grinding liver samples

6.1.2 Vortex mixer, VWR, Vortex Genie 2

6.1.3 Centrifuge, Mistral 1000 or IEC

6.1.4 Shaker, Eberbach or VWR

6.1.5 Nitrogen Evaporator, Organomation

6.1.6 Balance

7.0 Supplies and Materials

7.1 Gloves

7.2 Dissecting scalpels

7.3 Eppendorf or disposable pipettes

7.4 Nalgene bottles, capable of holding 250 mL and 1 L

7.5 Glass, type A, volumetric flasks

7.6 40 mL glass I-CHEM vials

7.7 Plastic sampule vials, Wheaton, 6 mL

7.8 Polypropylene centrifuge tubes, 15 mL

7.9 Labels

7.10 Syringes, capable of measuring 10 µL to 50 µL

7.11 Glass, type A, volumetric pipettes

7.12 Graduated pipettes

7.13 Electronic pipettor, Eppendorf or equivalent

7.14 Timer

7.15 Disposable plastic 3 cc syringes

7.16 Filters, nylon syringe filters, 0.2 µm, 25 mm

7.17 Crimp cap autovials

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 Reagents and Standards

8.1 Reagents

8.1.1 Sodium Hydroxide (J.T Baker or equivalent), (NaOH) 10N: weigh approximately 200 grams NaOH. Pour into a 1000 mL beaker containing 500 liters (L) Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L nalgene bottle.

8.1.2 Sodium Hydroxide (J.T Baker or equivalent), (NaOH) 1N. Dilute 10N 1:10. Measure 10 mL of the 10N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL nalgene bottle.

8.1.3 Tetrabutylammonium hydrogen sulfate (Kodak or equivalent), (TBA) 0.5M: Weigh approximately 169 grams of TBA into a 1 L volumetric containing 500 L Milli-Q™ water. Adjust to pH 10 using approximately 64 mL 10N NaOH and dilute to volume with Milli-Q™ water. Add NaOH slowly while adding the last 1 mL of NaOH because the pH changes abruptly. Store in a 1 L nalgene bottle.

8.1.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1N NaOH solution.

8.1.4 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker or equivalent), (Na_2CO_3/$NaHCO_3$) 0.25M: Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and dilute to volume with Milli-Q™ water. Store in a 1 L nalgene bottle.

8.1.5 PFOS (3M Specialty Chemical Division), molecular weight = 538.

8.1.6 Ethyl Acetate, Omnisolv, glass distilled or HPLC grade.

8.1.7 Methanol, Omnisolv, glass distilled or HPLC grade.

8.1.8 Liver and control liver, received frozen from testing laboratory.

8.1.9 Milli-Q™ water, all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus system.

8.2 Standards

8.2.1 Prepare PFOS standards for the standard curve.

8.2.2 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.

8.2.3 Bring to volume with methanol for a stock standard of approximately 1000 ppm (µg/mL).

8.2.4 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.2.5 Dilute the stock solution with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.2.6 Dilute the stock solution with methanol for a working standard 3 solution of approx. 0.50 ppm.

9.0 Sample Handling

9.1 All livers are received frozen and must be kept frozen until the extraction is performed.

10.0 Quality Control

10.1 Matrix Spikes

10.1.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.1.2 Prepare each spike using liver chosen by the analyst, usually a control liver.

10.1.3 Expected concentrations will fall in the mid-range of the initial calibration curve.

10.2 Continuing Calibration Checks

10.2.1 Prepare and analyze continuing calibration check samples to determine the continued linearity of the initial calibration curve.

10.2.2 One check is prepared per group of ten samples. For example, if a sample set = 34, four checks are prepared and extracted.

10.2.3 Prepare each continuing calibration check from the same liver homogenate used to prep the initial curve.

10.2.4 The expected concentration will fall within the mid-range of the initial calibration curve.

11.0 Calibration and Standardization

11.1 Prepare Liver Homogenate to Use for Standards

11.1.1 Weigh approximately 40 g of liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q™ water. Grind to a homogeneous solution.

11.1.2 If 40 g is not available, use appropriate amounts of liver and water in keeping with a 1:5 ratio.

11.1.3 See section 13.0 to calculate the actual density of liver.

11.1.4 Add 1 mL of homogeneous solution to a 15 mL centrifuge tube. Re-suspend homogeneous solution by shaking between aliquots while preparing a total of sixteen 1 mL aliquots of homogeneous solution in 15 mL centrifuge tubes.

11.1.5 Two 1 mL aliquots serve as matrix blanks. Use the standard concentrations and spiking amounts listed in table 1 to spike, in duplicate, two standard curves for a total of fourteen samples.

Table 1
Approximate Spiking Amounts for Calibration Standards

Working Standard (Approx. Conc.)	µL	Approx. final conc. of PFOS in liver
-	-	Blank
0.50 ppm	4	0.010 ppm
0.50 ppm	20	0.050 ppm
0.50 ppm	40	0.100 ppm
5.0 ppm	10	0.250 ppm
5.0 ppm	20	0.500 ppm
5.0 ppm	30	0.750 ppm
50 ppm	4	1.000 ppm

11.1.1 See section 13.0 to calculate actual concentrations of PFOS in calibration standards.

11.2 Extract spiked liver homogenates following **12.14-12.24** of this method. Use these standards to establish each initial curve on the mass spectrometer.

12.0 PROCEDURES

12.1 Obtain frozen liver samples. In spent tissue, note that the liver has not been packaged with other tissues.

12.2 Cut approximately 1 g of liver using a dissecting scalpel.

12.3 Weigh the sample directly into a tared plastic sampule vial.

12.4 Record the liver weight in the study notebook.

12.5 Label the sampule vial with the study number, weight, liver ID, date and analyst initials.

12.6 Add 2.5 mLs of water to sampule vial.

12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.

12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.

12.9 Take the grinder apart and clean it with methanol after each sample. Follow AMDT-EP-22.

12.10 Cap the sample and vortex for 15 seconds.

12.11 Pipette 1 mL homogenate into a 15 mL polypropylene centrifuge tube. Label the centrifuge tube with the identical information as the sampule vial. (See Worksheet for documenting the remaining steps.)

12.12 Spike liver homogenates with the appropriate amount of PFOS standard as described in section 11.1 or Table 1.

12.13 Pipette two 1 mL aliquots of Milli-Q™ water to centrifuge tubes. These will serve as instrument blanks.

12.14 Add 1 mL 0.5 M TBA and 2 mL of the 0.25 M sodium carbonate/sodium bicarbonate buffer.

12.15 Using a volumetric pipette, add 5 mLs ethyl acetate.

12.16 Cap each sample and put on the shaker for 20 minutes.

12.17 Centrifuge for 20 to 25 minutes, until layers are well separated. Set power on the centrifuge to approximately 3500 rpm.

12.18 Remove 4 mLs of organic layer, using a 5 mL graduated glass pipette, to a clean 15 mL centrifuge tube. Label this fresh tube with the same information as in **12.5**.

12.19 Put each sample on the analytical nitrogen evaporator until dry, approximately 2 to 3 hours.

12.20 Add 1.0 mL of methanol to each centrifuge tube using a graduated pipette.

12.21 Vortex mix for 30 seconds.

12.22 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial.

12.23 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) who performed the extraction.

12.24 Cap and hold for electrospray mass spectrometry analysis.

12.25 Complete the worksheet and tape to page of study notebook.

13.0 Data Analysis and Calculations

13.1 Calculations:

13.1.1 Calculate the density of liver (mg) in 1.0 mL homogenate using the following equation:

$$\frac{\text{g of Liver} \times \text{Average weight of ten 1 mL aliquots (mg)}}{(\text{g of Liver} + \text{g of Water})}$$

13.1.2 Calculate actual concentrations of PFOS in calibration standards using the following equation:

$$\frac{\mu L \text{ of Standard} \times \text{Concentration } (\mu g/mL)}{\text{mg Liver}^{*} / 1 \text{ mL homogenate}} = \text{Final Concentration } (\mu g/g \text{ or mg/kg}) \text{ of PFOS in Liver}$$

*Average weight of liver in solution as determined in **13.1.1**, by weighing ten 1 mL homogenates of approximately 40 mg liver in 200 mL of Milli-Q water.

14.0 Method Performance

14.1 The method detection limit is equal to half the lowest standard in the calibration curve.

15.0 Pollution Prevention and Waste Management

15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 Records

16.1 Complete the extraction worksheet and tape into the study notebook.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 The validation report associated with this method is FACT-M-1.0 & 2.0-V-1.

18.0 References

18.1 AMDT-EP-22, "Routine Maintenance of Ultra-Turrax T-25"

19.0 Affected Documents

19.1 FACT-M-2, "Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 Revisions

Revision Number.	Reason For Revision	Revision Date

Extraction Worksheet for FACT-M-1

Study #	Sample Number set #	PFOS approx. 0.5 ppm actual ppm #W____	PFOS approx. 5 ppm actual ppm #W____	PFOS approx. 50 ppm actual ppm #W____	Date and Initials for Std.
-	H_2O Blank	-	-	-	
-	____ Liver Blank	-	-	-	
-					
-					
-					
-					
-					
-					
-					
-					
1		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

[1] Study number where the original worksheet is located.

Blank Liver Homogenate: Std.#	Liver amount = g	
Liver Extraction Method :		**Date & Initials**
Vortex 15 sec.		
Pipette 1 mL of Liver Solution		
Pipette 1 mL of †0.5 M TBA, pH 10.	Std. #____	
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer	Std. #____	
Pipette 5 mL of Ethyl Acetate	TN-A-____	
Shake 20 min.		
Centrifuge 20-25 min. Centrifuge	Speed	
Remove a 4 mL aliquot of organic layer		
Put on Nitrogen Evaporator to dryness Evaporator	Temperature	
Add 1.0 mL of Methanol	TN-A-____	
Vortex 30 sec.		
Filter using a 3cc B-D syringe with a 0.2µm SRI filter into a 1.5 mL autosample vial		

MS/MSD/___ Cont. Checks: Spiked _____ uL of a _____ ppm std (____________) for a final concentration of ________ ppm. MS/MSD used sample ____________. Cont. Checks used same homogenate as for std curve.

3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICAL COMPOUNDS FROM SERUM OR OTHER FLUID FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: FACT-M-3.1

Adoption Date: 04/22/98

Revision Date: 10/01/98

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager — Date: 10/1/98

Group Leader — Date: 9/28/98

Lisa A Clemen
Technical Reviewer — Date: 9/28/98

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from serum or other fluid.

1.2 Applicable compounds: Fluorochemical surfactants or other fluorinated compounds.

1.3 Matrices: Rabbit, rat, bovine, and monkey serum, rat whole blood, and rat milk curd.

2.0 SUMMARY OF METHOD

2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemicals from serum, blood, or milk curd using an ion pairing reagent and 5.0 ml of ethyl acetate. In this method, seven fluorochemicals were extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, POAA, PFOSEA, and FC-807 monoester (see *3.0 **Definitions***). An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into ethyl acetate. Four ml of extract are removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 ml of methanol, then filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

3.0 DEFINITIONS

3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3^-$

3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$

3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$

3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$

3.5 POAA: perfluorooctanoate (anion of ammonium salt) $C_7F_{15}COO^-$

3.6 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$

3.7 FC-807 monoester $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2O\text{-}PO_3H)$

3.8 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

4.0 WARNINGS AND CAUTIONS

4.1 **Health and safety warnings**

4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

5.0 INTERFERENCES

5.1 There are no known interferences at this time.

6.0 EQUIPMENT

6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.

6.1.1 Vortex mixer, VWR, Vortex Genie 2

6.1.2 Centrifuge, Mistral 1000 or IEC

6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

6.1.5 Balance (± 0.100 g)

7.0 Supplies and Materials

7.1 Gloves

7.2 Eppendorf or disposable pipettes

7.3 Electronic pipettor, Eppendorf or equivalent

7.4 Graduated pipettes

7.5 Nalgene bottles, capable of holding 250 mL and 1 L

7.6 Volumetric flasks, glass, type A

7.7 Volumetric pipets, glass, type A

7.8 I-CHEM vials, glass, 40 mL glass

7.9 Crimp cap autovials

7.10 Centrifuge tubes, polypropylene, 15 mL

7.11 Labels

7.12 Syringes, capable of measuring 5 µL to 50 µL

7.13 Syringes, disposable plastic, 3 cc

7.14 Syringe filters, nylon, 0.2 µm, 25 mm

7.15 Timer

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 Reagents and Standards

8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system

8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

8.4 Sodium carbonate (Na_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), J.T. Baker or equivalent

8.6 Ethyl acetate, Omnisolv, glass distilled or HPLC grade

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Serum or blood, frozen from supplier

8.9 Control matrix or blank matrix for purpose of standards, QC checks, blanks, etc.

8.10 **Fluorochemical standards**

8.10.1 PFOS (3M Specialty Chemical Division), molecular weight = 538

8.10.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

8.10.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585

8.10.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 571

8.10.5 POAA (3M Specialty Chemical Division), molecular weight = 431

8.10.6 PFOSEA (3M Specialty Chemical Division), molecular weight = 527

8.10.7 FC-807 monoester (3M Specialty Chemical Division). FC-807 is a mixture of triester, diester, and monoester fluorochemical components. The monoester molecular weight = 650

8.10.8 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428

8.10.9 Other fluorochemicals, as appropriate

8.11 Reagent preparation

8.11.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.11.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.

8.11.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH and dilute to volume with Milli-Q™ water. While adding the last mL of NaOH, add slowly because the pH changes abruptly. Store in a 1 L Nalgene bottle.

8.11.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.

8.11.4 0.25 M sodium carbonate/sodium bicarbonate buffer ($Na_2CO_3/NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.12 Standards preparation

8.12.1 Prepare PFOS standards for the standard curve.

8.12.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)

8.12.3 Weigh approximately 100 mg of PFOS into a 100 ml volumetric flask and record the actual weight.

8.12.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm (µg/ml).

8.12.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.12.6 Dilute the stock solution with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.12.7 Dilute the stock solution with methanol for a working standard 3 solution of approx. 0.50 ppm.

8.13 Surrogate stock standard preparation

8.13.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{13}SO_3H$ into a 50 ml volumetric flask and record the actual weight.

8.13.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.13.3 Prepare a surrogate working standard. Transfer approximately 0.5 ml of surrogate stock to a 50 ml volumetric flask and bring to volume with methanol for a working standard of 10-20 ppm. Record the actual volume transferred.

9.0 Sample Handling

9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

10.0 Quality Control

10.1 Matrix blanks and method blanks

10.1.1 Extract two 1.0 mL aliquots of the appropriate matrix (serum or blood, with blood samples diluted 1:1 with Milli-Q™ water) following this procedure and use as matrix blanks. See **11.1.4**.

10.1.2 Extract two 1.0 ml aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.2 Matrix spikes

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually the control matrix received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

10.3 Continuing calibration checks

10.3.1 Prepare and analyze continuing calibration check samples to ensure the accuracy of the initial calibration curve. If the percent difference between the initial curve and the continuing check differ by >30%, re-analyze samples analyzed after the last acceptable check.

10.3.2 Prepare one check per group of ten samples. For example, if a sample set = 34, prepare and extract four checks.

10.3.3 Prepare each continuing calibration check from the same matrix used to prepare the initial curve.

10.3.4 The expected concentration will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

Note: Blood coagulates in air; therefore, minimize air contact until dilution. At this point, add TBA and buffer to each centrifuge tube as in step 12.9, then add 1.0 mL of the diluted matrix sample to each tube.

11.1.1 Transfer 1 mL of serum or 1 mL of blood (blood is diluted 1:1 with Milli-Q™ water) to a 15 mL centrifuge tube. The blood is similar in composition to milk curd and can be used in place of milk curd for standard curves when extracting that matrix.

11.1.2 If most sample volumes are less than 1.0 mL, extract standards with matrix volumes equal to the sample volumes. Do not extract below 0.50 mL of matrix. Record the sample volume on the extraction sheet.

11.1.3 While preparing a total of twenty aliquots in 15 ml centrifuge tubes, mix or shake between aliquots.

11.1.4 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen standards and two matrix blanks.

11.1.5 Refer to validation reports **FACT-M-3.1-V-1** and **FACT-M-4.1-V-1,** which list the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.6 Use Attachment D as an aid in calculating the concentrations of the working standards. See Section **13.0** to calculate actual concentrations of PFOS in calibration standards.

11.2 To each standard, blank, or QC check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb -1000 ppb.

11.3 Extract spiked matrix standards following **12.6-12.16** of this method. Use these standards to establish each initial curve on the mass spectrometer.

Table 1 **Approximate spiking amounts for standards and spikes using 1.0 ml of matrix**		
Working standard (approx. conc.)	µL	Approx. final conc. of analyte in matrix
-	-	Blank
0.500 ppm	10	0.005 ppm
0.500 ppm	20	0.010 ppm
5.00 ppm	5	0.025 ppm
5.00 ppm	10	0.050 ppm
5.00 ppm	20	0.100 ppm
50.0 ppm	5	0.250 ppm
50.0 ppm	10	0.500 ppm
50.0 ppm	15	0.750 ppm
50.0 ppm	20	1.00 ppm

Table 2 **Approximate spiking amounts for standards and spikes using 0.5 ml of matrix**		
Working standard (approx. conc.)	µL	Approx. final conc. of analyte in matrix
-	-	Blank
0.500 ppm	5	0.005 ppm
0.500 ppm	10	0.010 ppm
5.00 ppm	2.5	0.025 ppm
5.00 ppm	5	0.050 ppm
5.00 ppm	10	0.100 ppm
50.0 ppm	2.5	0.250 ppm
50.0 ppm	5	0.500 ppm
50.0 ppm	7.5	0.750 ppm
50.0 ppm	10	1.00 ppm

12.0 PROCEDURE

12.1 Obtain frozen samples and allow to thaw.

12.2 Vortex mix for 15 seconds, then transfer 1.0 mL or other appropriate volume to a 15 mL polypropylene centrifuge tube. For blood samples, remove 0.5 mL and dilute to 1.0 mL with Milli-Q™ water. As soon after diluting as possible, pipet diluted blood into TBA-buffer mixture shown in step 12.9 and mix well.

12.3 Return samples to freezer after extraction amount has been removed.

12.4 Record the volume on the extraction worksheet. The final methanol volume equals the volume transferred from the sample. For example, if 0.5 mL is removed for a blood sample, the final methanol volume will equal 0.5 mL.

12.5 Label the tube with the study number, sample ID, date and analyst initials. See attached worksheet for documenting the remaining steps.

12.6 Spike each matrix with the appropriate amount of standard as described in **11.1** or **Table 1** or **2** in that section for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.

12.7 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in **11.2**.

12.8 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.

12.9 To each sample, add 1 mL 0.5 M TBA and 2 mL of 0.25 M sodium carbonate/sodium bicarbonate buffer.

12.10 Using a volumetric pipette, add 5 mL ethyl acetate.

12.11 Cap each sample and put on the shaker for 20 minutes.

12.12 Centrifuge for 20 to 25 minutes at approximately 3500 rpm, until layers are well separated.

12.13 Transfer 4 mL of organic layer, using a 5 mL graduated glass pipette, to a clean 15 mL centrifuge tube. Label this fresh tube with the same information as in 12.5.

12.14 Put each sample on the analytical nitrogen evaporator until dry, approximately 2 to 3 hours.

12.15 Add 1.0 mL or other appropriate volume of methanol to each centrifuge tube using a graduated pipette. Methanol volume to add equals the initial volume of sample used for the extraction.

12.16 Vortex mix for 30 seconds.

12.17 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.

12.18 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.

12.19 Cap and store extracts at approximately 4 °C until analysis.

12.20 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

13.0 Data Analysis and Calculations

13.1 Calculations

13.1.1 Calculate actual concentrations of PFOS, or other applicable fluorochemical, in calibration standards using the following equation:

$$\frac{\text{mL of standard x concentration of standard } (\mu g/mL)}{\text{mL of standard + mL of surrogate standard + initial matrix volume (mL)}} =$$

Final Concentration (µg/mL) of PFOS in matrix

14.0 Method Performance

14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).

14.2 The following quality control samples are extracted with each batch of samples to ensure the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks

14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction

14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve

15.0 Pollution Prevention and Waste Management

15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 Records

16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in study 3-ring binder, as appropriate.

17.0 Attachments

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values

17.3 Attachment C, LOQ Summary

17.4 Attachment D, Calibration standard concentration worksheet

18.0 References

18.1 The validation reports associated with this method are **FACT-M-3.1 & 4.1-V-1**.

BACK TO MAIN

19.0 Affected Documents

19.1 FACT-M-4.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 Revisions

Revision Number	Reason For Revision	Revision Date
1	Validation of method to include 7 fluorochemicals, an additional matrix, new API/MS(MS) systems, monkey serum cross validation, improvements to ion pairing extraction, MDL study, updates in record keeping and storing policies, etc.	07/01/98

Study #	Sample Number set #	FC-Mix approx. 0.5 ppm actual ppm #W____	FC-Mix approx. 5 ppm actual ppm #W____	FC-Mix approx. 50 ppm actual ppm #W____	Date and Initials for Std. or Comments
-	H_2O Blank	-	-	-	
-	____ Blank	-	-	-	
-					
-					
-					
-					
-					
-					
-					
[1]		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

[1] Study number where the original worksheet is located.

Blank Std # amount = mL

Serum Extraction Method : **Date & Initials**

Vortex 15 sec.

Pipette Matrix Volume ml

Pipette 1 ml of 0.5 M TBA, pH 10. Std. #____

Pipette 2 ml of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer Std. #____

Pipette 5 ml of ethyl acetate TN-A-____

Shake 20 min.

Centrifuge 20-25 min. Centrifuge speed:

Remove a 4 ml aliquot of organic layer

Put on Nitrogen Evaporator to dryness Evaporator #: Temperature:

Add methanol Volume ____ ml TN-A-____

Vortex 30 sec.

Filter using a 3cc B-D syringe with a 0.2µm SRI filter into a 1.5 ml autosample vial

MS/MSD/___ Cont. Checks: Spiked ____ uL of a ____ ppm std (________) for a final concentration of ______ ppm. MS/MSD used sample ________. Cont. Checks used same matrix as for std curve.

Surrogate Standard: Spiked ____ uL of a ____ ppm std (________) to all samples, standards, and blanks

MDL/LOQ values for Rabbit Serum:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	1.38	4.39	5 ppb – 1000 ppb
PFOSA	2.23	7.09	10 ppb – 1000 ppb
PFOSAA	2.84	9.04	10 ppb – 1000 ppb
EtFOSE-OH	3.90	12.4	15 ppb – 1000 ppb
POAA	4.31	13.7	15 ppb – 750 ppb
PFOSEA	1.09	3.48	25 ppb – 1000 ppb
Monoester	149	248	MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for monoester will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.

MDL/LOQ values for Rat Serum:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	1.27	4.04	10 ppb – 1000 ppb
PFOSA	2.14	6.81	25 ppb – 1000 ppb
PFOSAA	2.32	7.38	10 ppb – 1000 ppb
EtFOSE-OH	3.25	10.3	50 ppb – 1000 ppb
POAA	1.20	3.81	5 ppb – 1000 ppb
PFOSEA	1.84	5.86	10 ppb – 1000 ppb
Monoester	149	248	MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for monoester will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.

MDL/LOQ values for Bovine Serum:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	2.11	6.70	25 ppb – 1000 ppb
PFOSA	5.04	16.0	25 ppb – 1000 ppb
PFOSAA	2.34	7.45	260 ppb – 1000 ppb
EtFOSE-OH	11.3	35.8	50 ppb – 1000 ppb
POAA	4.64	14.8	15 ppb – 1000 ppb
PFOSEA	3.71	11.8	15 ppb – 1000 ppb
Monoester	149	248	MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for monoester will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.

No data is available for MDL or LOQ in Monkey Serum. Use validated Linear Calibration Range instead.
Please see Attachment C (LOQ Summary) and MDL study in FACT-M-3.1 & 4.1-V-1 for specifics.

MDL/LOQ values for Monkey Serum:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	1.38	4.39	MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for PFOS will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.
PFOSA	2.23	7.09	MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for PFOSA will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.
PFOSAA	2.84	9.04	MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for PFOSAA will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.
EtFOSE-OH	3.90	12.4	MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for EtFOSE-OH will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.
POAA	4.31	13.7	MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for POAA will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.
PFOSEA	1.09	3.48	MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for PFOSEA-OH will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.
Monoester	149	248	MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for EtFOSE-OH will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.

MDL/LOQ values for Rat Whole Blood:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate Concentrations to be used for preparing the Standard Calibration Curve
PFOS	1.25	3.96	5 ppb – 1000 ppb
PFOSA	1.77	5.65	10 ppb – 1000 ppb
PFOSAA	17.3	55.0	55 ppb – 1000 ppb
EtFOSE-OH	7.89	25.1	MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for EtFOSE-OH will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.
POAA	4.73	15.1	15 ppb – 1000 ppb
PFOSEA	24.2	77.1	80 ppb – 1000 ppb
Monoester	58.0	185	MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for monoester will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.

Please see Attachment C (LOQ Summary) and MDL study in FACT-M-3.1 & 4.1-V-1 for specifics.

Ion Pairing Extraction of Fluorochemicals from Serum and Analysis by API/MS(MS)
Summary Table: Limits of Quantitation

Compound	Matrix	MDL	LOQ	Approximate linear range[2] Low std	High std
PFOS	Rabbit	1.38 ppb	4.39 ppb	5 ppb	1000 ppb
	Bovine	2.11 ppb	6.70 ppb	25 ppb	1000 ppb
	Rat	1.27 ppb	4.04 ppb	10 ppb	1000 ppb
	Monkey	n/d	n/d	25 ppb	1000 ppb
PFOSA	Rabbit	2.23 ppb	7.09 ppb	10 ppb	1000 ppb
	Bovine	5.04 ppb	16.0 ppb	25 ppb	1000 ppb
	Rat	2.14 ppb	6.81 ppb	25 ppb	1000 ppb
	Monkey	n/d	n/d	25 ppb	1000 ppb
PFOSAA	Rabbit	2.84 ppb	9.04 ppb	10 ppb	1000 ppb
	Bovine	2.34 ppb	7.45 ppb	263 ppb	1000 ppb
	Rat	2.32 ppb	7.38 ppb	10 ppb	1000 ppb
	Monkey	n/d	n/d	25 ppb	1000 ppb
EtFOSE-OH	Rabbit	3.90 ppb	12.4 ppb	15 ppb	1000 ppb
	Bovine	11.3 ppb	35.8 ppb	50 ppb	1000 ppb
	Rat	3.25 ppb	10.3 ppb	50 ppb	1000 ppb
	Monkey	n/d	n/d	10 ppb	1000 ppb
POAA	Rabbit	4.31 ppb	113.7 ppb	15 ppb	750 ppb
	Bovine	4.64 ppb	14.8 ppb	5 ppb	1000 ppb
	Rat	1.20 ppb	3.81 ppb	5 ppb	1000 ppb
	Monkey	n/d	n/d	5 ppb	1000 ppb
PFOSEA	Rabbit	1.03 ppb	3.48 ppb	25 ppb	1000 ppb
	Bovine	3.71 ppb	11.8 ppb	5 ppb	1000 ppb
	Rat	1.84 ppb	5.86 ppb	10 ppb	1000 ppb
	Monkey	n/d	n/d	5 ppb	1000 ppb
Monoester[1]	Rabbit	149 ppb	474.0 ppb	250 ppb	1000 ppb
	Bovine	149 ppb	474.0 ppb	250 ppb	1000 ppb
	Rat	149 ppb	474.0 ppb	250 ppb	1000 ppb
	Monkey	n/d	n/d	100 ppb	1000 ppb

1. Values for monoester are estimates only.
2. Highest standard (approx. 1500 ppb) was excluded from final LCR and upper LOQ values due to poor R & R values and excessive weighting of the calibration curve.

Compound: PFOS

Serum matrix	Prepared range of standards (ppb)(ng/mL)	Range of average curve (ppb)(ng/mL)	LCR from ave curve (ppb)(ng/mL)	Range of low std curve (ppb)(ng/mL)	LCR from low std curve (ppb)(ng/mL)	Range of high std curve (ppb)(ng/mL)	LCR from high std curve (ppb)(ng/mL)
Rabbit	4.93 - 1450	4.93 - 1450	49.3 - 1000	49.3 - 97.6	4.93 - 97.6	97.6 - 1450	97.6 - 1000
Bovine	4.93 - 1450	4.93 - 1450	97.6 - 1000	4.93 - 248	24.8 - 248	97.6 - 1450	97.6 - 1000
Rat	4.93 - 1450	4.93 - 976	24.8 - 976	4.93 -248	9.76 - 248	97.6 - 1450	248 - 1000
Monkey	4.93 - 1450	4.93 - 1450	24.8 - 1000	24.8 -493	24.8 - 493	97.6 - 1450	97.6 - 1000

Compound: PFOSA

Serum matrix	Prepared range of standards (ppb)(ng/mL)	Range of average curve (ppb)(ng/mL)	LCR from ave curve (ppb)(ng/mL)	Range of low std curve (ppb)(ng/mL)	LCR from low std curve (ppb)(ng/mL)	Range of high std curve (ppb)(ng/mL)	LCR from high std curve (ppb)(ng/mL)
Rabbit	5.00 - 1470	5.00 - 1470	9.89 – 1000	5.00 – 251	n/a	98.9 – 1470	98.9 – 1000
Bovine	5.00 - 1470	5.00 - 1470	25.1 – 1000	5.00 – 98.9	n/a	98.9 – 1470	98.9 – 1000
Rat	5.00 - 1470	5.00 - 1470	50.0 – 1000	9.89 – 500	25.1 – 500	98.9 – 1470	98.9 – 1000
Monkey	5.00 - 1470	5.00 - 1470	98.9 – 1000	25.1 - 500	25.1 - 500	98.9 – 1470	n/a

Compound: PFOSAA

Serum matrix	Prepared range of standards (ppb)(ng/mL)	Range of average curve (ppb)(ng/mL)	LCR from ave curve (ppb)(ng/mL)	Range of low std curve (ppb)(ng/mL)	LCR from low std curve (ppb)(ng/mL)	Range of high std curve (ppb)(ng/mL)	LCR from high std curve (ppb)(ng/mL)
Rabbit	5.20 – 1540	5.20 – 1540	104 – 1000	5.20 – 263	10.4 – 263	104 – 1540	263 – 1000
Bovine	5.20 – 1540	5.20 – 1540	263 – 1000	10.4 – 521	n/a (1)	104 – 1540	263 – 1000
Rat	5.20 – 1540	5.20 – 1540	104 – 1000	5.20 – 263	10.4 – 263	104 – 1540	263 – 1000
Monkey	5.20 – 1540	5.20 – 1540	52.4 – 1000	5.20 – 263	26.3 – 263	104 – 1540	263 – 1000

Compound: EtFOSE-OH

Serum matrix	Prepared range of standards (ppb)(ng/mL)	Range of average curve (ppb)(ng/mL)	LCR from ave curve (ppb)(ng/mL)	Range of low std curve (ppb)(ng/mL)	LCR from low std curve (ppb)(ng/mL)	Range of high std curve (ppb)(ng/mL)	LCR from high std curve (ppb)(ng/mL)
Rabbit	4.94 - 1450	4.94 - 1450	49.4 - 1000	4.94 – 248	9.78 – 248	97.8 - 1450	n/a
Bovine	4.94 - 1450	4.94 - 1450	97.8 - 1000	4.94 – 248	4.94 – 248	97.8 - 1450	248 - 1000
Rat	4.94 - 1450	4.94 - 1450	494 – 1000	4.94 – 248	n/a	97.8 - 1450	97.8 - 1000
Monkey	4.94 - 1450	4.94 -1450	97.8 - 1000	4.94 – 248	9.78 - 248	97.8 - 1450	n/a

Compound: POAA

Serum matrix	Prepared range of standards (ppb)(ng/mL)	Range of average curve (ppb)(ng/mL)	LCR from ave curve (ppb)(ng/mL)	Range of low std curve (ppb)(ng/mL)	LCR from low std curve (ppb)(ng/mL)	Range of high std curve (ppb)(ng/mL)	LCR from high std curve (ppb)(ng/mL)
Rabbit	5.01 – 1480	5.13 – 1510	25.8 – 1000	5.13 – 258	n/a	102 – 1510	n/a
Bovine	5.01 – 1480	5.13 – 1510	102 – 1000	5.13 – 258	5.13 – 258	102 – 1510	258 – 1000
Rat	5.01 – 1480	5.13 – 1510	51.3 – 1000	5.13 – 102	5.13 – 102	102 – 1510	102 – 1000
Monkey	5.01 – 1480	5.13 – 1510	102 – 1000	5.13 – 102	5.13 – 102	102 – 1510	258 – 1000

Compound: PFOSEA

Serum matrix	Prepared range of standards (ppb)(ng/mL)	Range of average curve (ppb)(ng/mL)	LCR from ave curve (ppb)(ng/mL)	Range of low std curve (ppb)(ng/mL)	LCR from low std curve (ppb)(ng/mL)	Range of high std curve (ppb)(ng/mL)	LCR from high std curve (ppb)(ng/mL)
Rabbit	5.13 - 1510	5.13 - 1510	25.8 - 1000	5.13 – 258	n/a	102 - 1510	n/a
Bovine	5.13 - 1510	5.13 - 1510	102 - 1000	5.13 – 258	5.13 – 258	102 - 1510	258 – 1000
Rat	5.13 - 1510	5.13 - 1510	51.3 – 1000	5.13 – 102	5.13 – 102	102 - 1510	102 – 1000
Monkey	5.13 - 1510	5.13 - 1510	102 – 1000	5.13 – 102	5.13 – 102	102 - 1510	258 – 1000

Compound: Monoester

Serum matrix	Prepared range of standards (ppb)(ng/mL)	Range of average curve (ppb)(ng/mL)	LCR from ave curve (ppb)(ng/mL)
Rabbit	4.94 - 1450	9.78 – 978	n/a
Bovine	4.94 - 1450	97.8 – 1450	n/a
Rat	4.94 - 1450	248 – 1450	248 – 1000
Monkey	4.94 - 1450	49.4 - 1450	97.8 – 1000

In general, the chromatography for the monoester was very poor (broad peaks, high baseline).
Curves for monoester in rabbit and bovine were unacceptable. Any quantitation performed with the monoester is only an estimate and should not be used for reliable, accurate data reporting.

BACK TO MAIN

Ion Pair Standard Curves – Fluids

Prep date(s):
Analyte(s):
Sample matrix:

Standard number:
Equipment number:
Final solvent and TN:
Blank fluid/identifier:

Method/revision:
Target analyte(s):
FC mix std approx. 0.500 ppm: W398-641
FC mix std approx. 5.00 ppm: W398-640
FC mix std approx. 50.0 ppm: W398-639
Surrogate std approx. 17.71 ppm: W398-605

Actual concentrations of standards in the FC mix

PFOS Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE-OH Std conc ug/mL	POAA Std conc ug/mL	PFOSEA Std conc ug/mL	Monoester Std conc ug/mL	All Am't spiked mL	All Final Volume mL
0.500	0.507	0.532	0.501	0.509	0.521	0.501	0.010	1.015
0.500	0.507	0.532	0.501	0.509	0.521	0.501	0.020	1.025
5.00	5.07	5.32	5.01	5.09	5.21	5.01	0.005	1.010
5.00	5.07	5.32	5.01	5.09	5.21	5.01	0.010	1.015
5.00	5.07	5.32	5.01	5.09	5.21	5.01	0.020	1.025
50.0	50.1	53.2	50.1	50.9	52.1	50.1	0.005	1.010
50.0	50.1	53.2	50.1	50.9	52.1	50.1	0.010	1.015
50.0	50.1	53.2	50.1	50.9	52.1	50.1	0.015	1.020
50.0	50.1	53.2	50.1	50.9	52.1	50.1	0.020	1.025

Calculated concentrations of standards in the sample matrix

PFOS Final conc ng/mL	PFOSA Final conc ng/mL	PFOSAA Final conc ng/mL	EtFOSE Final conc ng/mL	POAA Final conc ng/mL	PFOSEA Final conc ng/mL	Monoester Std conc ng/mL	Surrogate Std conc ng/mL	All Am't spiked (mL)
4.93	5.00	5.24	4.94	5.01	5.13	4.94	2.64	0.005
9.76	9.89	10.4	9.78	9.93	10.2	9.78		
24.8	25.1	26.3	24.8	25.2	25.8	24.8	**Surrogate**	
49.3	50.0	52.4	49.4	50.1	51.3	49.4	Final conc	
97.6	98.9	104	97.8	99.3	102	97.8	ng/mL	
248	251	263	248	252	258	248	81.0	
493	500	524	494	501	513	494		
735	746	782	737	749	766	737		
976	989	1038	978	993	1017	978		

Validated ranges – approximate concentrations

Sera	PFOS	PFOSA	PFOSAA	EtFOSE-OH	POAA	PFOSEA
Rabbit	5-1000 ppb	10-1000 ppb	10-1000 ppb	10-1000 ppb	10-750 ppb	25-1000 ppb
Bovine	25-1000 ppb	25-1000 ppb	263-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb
Rat	10-1000 ppb	25-1000 ppb	10-1000 ppb	50-500 ppb	5-1000 ppb	5-1000 ppb
Monkey	Estimates only.	Use values for	Rabbit			

3M Environmental Laboratory

Method

Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

Method Number: ETS-8-4.1

Adoption Date: 03/01/99

Revision Date: 4/27/99

Author: Lisa Clemen, Glenn Langenburg

Approved By:

[signature]	4/27/99
Laboratory Manager	Date
[signature]	4/26/99
Group Leader	Date
Lisa A Clemen	04/26/99
Technical Reviewer	Date

1.0 Scope and Application

1.1 Scope: This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from serum.

1.2 Applicable compounds: Fluorochemical surfactants or other fluorinated compounds.

1.3 Matrices: Rabbit, rat, bovine, monkey, and human serum or other fluids as designated in the validation report.

2.0 SUMMARY OF METHOD

2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from serum, or other fluids, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals were extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard (see *3.0 Definitions*). An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL of methanol, then filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

2.2 These sample extracts are analyzed following method ETS-8-5.1 or other appropriate methods.

3.0 DEFINITIONS

3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3^-$

3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$

3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$

3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$

3.5 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$

3.6 M556: $C_8F_{17}SO_2N(H)(CH_2COOH)$

3.7 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

4.0 WARNINGS AND CAUTIONS

4.1 Health and safety warnings

4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

5.0 INTERFERENCES

5.1 There are no interferences known at this time.

6.0 EQUIPMENT

6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.

6.1.1 Vortex mixer, VWR, Vortex Genie 2

6.1.2 Centrifuge, Mistral 1000 or IEC

6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

6.1.5 Balance (± 0.100 g)

7.0 SUPPLIES AND MATERIALS

7.1 Gloves

7.2 Eppendorf or disposable pipettes

7.3 Nalgene bottles, capable of holding 250 mL and 1 L

7.4 Volumetric flasks, glass, type A

7.5 I-CHEM vials, glass, 40 mL glass

7.6 Centrifuge tubes, polypropylene, 15 mL

7.7 Labels

7.8 Oxford Dispenser – 3.0 to 10.0 mL

7.9 Syringes, capable of measuring 5 µL to 50 µL

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 cc

7.12 Syringe filters, nylon, 0.2 µm, 25 mm

7.13 Timer

7.14 Crimp cap autovials and caps

7.15 Crimpers

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 REAGENTS AND STANDARDS

8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system

8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

8.4 Sodium carbonate (Na_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), J.T. Baker or equivalent

8.6 Methyl-T-Butyl Ether, Omnisolv, glass distilled or HPLC grade

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Serum or blood, frozen from supplier

8.9 **Fluorochemical standards**

8.9.1 PFOS (3M Specialty Chemical Division), molecular weight = 538

8.9.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

8.9.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585

8.9.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570

8.9.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527

8.9.6 M556 (3M Specialty Chemical Division), molecular weight = 557

8.9.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428

8.9.8 Other fluorochemicals, as appropriate

8.10 Reagent preparation

NOTE: When preparing larger volumes than listed in reagent, standard, or surrogate preparation, adjust accordingly.

8.10.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.10.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.

8.10.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.10.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.

8.10.4 0.25 M sodium carbonate/sodium bicarbonate buffer (Na_2CO_3/$NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.11 Standards preparation

8.11.1 Prepare PFOS standards for the standard curve.

8.11.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)

8.11.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.

8.11.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm (µg/mL).

8.11.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.11.6 Dilute working standard 1 with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.11.7 Dilute working standard 1 with methanol for a working standard 3 solution of approx. 0.50 ppm.

8.12 Surrogate stock standard preparation

8.12.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{13}SO_3H$ into a 50 mL volumetric flask and record the actual weight.

8.12.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.12.3 Prepare a surrogate working standard. Transfer approximately 1 mL of surrogate stock to a 10 mL volumetric flask and bring to volume with methanol for a working standard of 100 ppm. Record the actual volume transferred.

9.0 Sample Handling

9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

9.2 Allow samples to thaw to room temperature prior to extraction.

10.0 Quality Control

10.1 Solvent Blanks, Method blanks and matrix blanks

10.1.1 An aliquot of 1.0 mL methanol is used as a solvent blank.

10.1.2 Extract two 1.0 mL aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.1.3 Extract two 1.0 mL aliquots of the serum following this procedure and use as matrix blanks. See **11.1.4.**

10.2 Matrix spikes

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually the control matrix received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

10.3 Continuing calibration checks

10.3.1 Prepare continuing calibration check samples to ensure the accuracy of the initial calibration curve.

10.3.2 Prepare, at a minimum, one continuing check per group of 10 samples. For example, if a sample set = 34, four checks are prepared and extracted.

10.3.3 Prepare each continuing calibration check from the same matrix used to prepare the initial curve.

10.3.4 The expected concentrations will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than
5 ppb – 1000 ppb).

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

11.1.1 Transfer 1 mL of serum to a 15 mL centrifuge tube.

11.1.2 If most sample volumes are less than 1.0 mL, extract standards with matrix volumes equal to the sample volumes. Do not extract less than 0.50 mL of matrix. Record each sample volume on the extraction sheet.

11.1.3 While preparing a total of twenty aliquots in 15 mL centrifuge tubes, mix or shake between aliquots.

11.1.4 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen standards, two matrix blanks, and two method blanks.

11.1.5 Refer to validation report **ETS-8-4.0 & ETS-8-5.0-V-1,** which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.6 Use Attachment D as an aid in calculating the concentrations of the working standards. See Section **13.0** to calculate actual concentrations of PFOS in calibration standards.

11.2 To each standard, blank, or continuing check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb - 1000 ppb.

11.3 Extract spiked matrix standards following **12.6-12.16** of this method. Use these standards to establish each initial curve on the mass spectrometer.

Table 1 **Approximate spiking amounts for standards and spikes** **Using 1.0 mL of matrix**		
Working standard (approx. conc.)	µL	Approx. final conc. of analyte in matrix
-	-	Blank
0.500 ppm	10	0.005 ppm
0.500 ppm	20	0.010 ppm
5.00 ppm	5	0.025 ppm
5.00 ppm	10	0.050 ppm
5.00 ppm	20	0.100 ppm
50.0 ppm	5	0.250 ppm
50.0 ppm	10	0.500 ppm
50.0 ppm	15	0.750 ppm
50.0 ppm	20	1.00 ppm

12.0 PROCEDURE

12.1 Obtain frozen samples and allow to thaw at room temperature or in a lukewarm waterbath.

12.2 Vortex mix for 15 seconds, then transfer 1.0 mL or other appropriate volume to a 15 mL polypropylene centrifuge tube.

12.3 Return unused samples to freezer after extraction amounts have been removed.

12.4 Record the initial volume on the extraction worksheet.

12.5 Label the tube with the study number, sample ID, date and analyst initials. See attached worksheet for documenting the remaining steps.

12.6 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in **11.2.**

12.7 Spike each matrix with the appropriate amount of standard as described in **11.1**, or **Table 1** in that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.

12.8 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.

12.9 Check to ensure the 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.

12.10 To each sample, add 1 mL 0.5 M TBA and 2 mL of 0.25M sodium carbonate/sodium bicarbonate buffer.

12.11 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.

12.12 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.

12.13 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.

12.14 Label a fresh 15 mL centrifuge tube with the same information as in 12.5.

12.15 Remove 4.0 mL of the organic layer to this clean 15 mL centrifuge tube.

12.16 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.

12.17 Add 1.0 mL of methanol to each centrifuge tube using a graduated pipette.

12.18 Vortex mix for 30 seconds.

12.19 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.

12.20 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.

12.21 Cap and store extracts at room temperature or at approximately 4 °C until analysis.

12.22 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations

13.1.1 Calculate actual concentrations of PFOS, or other applicable fluorochemical, in calibration standards using the following equation:

$$\frac{\text{mL of standard x concentration of standard } (\mu g/mL)}{\text{mL of standard + mL of surrogate standard + initial matrix volume (mL)}} =$$

Final Concentration (µg/mL) of PFOS in matrix

14.0 METHOD PERFORMANCE

14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).

14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks.

14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.

14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve.

14.3 Refer to section 14 of ETS-8-5.1 for method performance criteria.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in the 3-ring study binder, as appropriate.

17.0 ATTACHMENTS

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard concentration worksheet

18.0 REFERENCES

18.1 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1.**

18.2 FACT-M-3.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

19.1 ETS-8-5.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 REVISIONS

Revision Number	Reason For Revision	Revision Date
1	Section 12.21 Changed to include sample storage at room temperature. Section 12.13 Added the shaker speed. Section 12.17 Final volume is 1.0 mL; not adjusted for initial volumes less than 1.0 mL.	04/02/99

Extraction Worksheet ETS-8-4.1

Study #________ Matrix________ Box #________ Wk/Day________ DateSpiked/Analyst	Surrogate Std approx. ppm actual ppm #________	FC-Mix approx. 0.5 pm actual ppm #________	FC-Mix approx. 5 ppm actual ppm #________	FC-Mix approx. 50 ppm actual ppm #________	Comments
CCV					
MS					
MSD					
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

Blank	Std #	amount =	mL
Serum Extraction Method	:		**Date & Initials**
Vortex 15 sec.			
Pipette Matrix	Volume	mL	
Pipette 1 mL of 0.5 M TBA, pH 10. pH = ________	Std. #________		
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer	Std. #________		
Dispense 5 mL of methyl-t-butyl ether	TN-A-________		
Shake 20 min.	Shaker speed:		
Centrifuge 20-25 min.	Centrifuge speed:		
Remove a 4 mL aliquot of organic layer			
Put on Nitrogen Evaporator to dryness	Temperature:		
Add methanol Volume________mL	TN-A-________		
Vortex 30 sec.			
Filter using a 3cc B-D syringe with a 0.2μm filter into a 1.5 mL autosample vial			

Cont. Cal. Verifications used same matrix as for std curve.

MDL/LOQ values for rabbit serum

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	1.74	5.55	5 ppb - 1000 ppb
PFOSA	1.51	4.79	5 ppb - 1000 ppb
PFOSAA	3.46	20.5	5 ppb - 1000 ppb
EtFOSE-OH	11.4	36.2	5 ppb - 1000 ppb
M556	6.03	19.2	5 ppb - 1000 ppb
PFOSEA	5.71	18.2	5 ppb - 1000 ppb

MDL/LOQ values in rat, bovine, monkey, and human serum, and monkey plasma were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the rabbit serum curves to determine equivalence. Responses in the rat, bovine, monkey, and human were equivalent to the rabbit responses, therefore, their MDL and LOQ will be the same values as determined in rabbit serum.

Please see LOQ Summary and MDL study in ETS-8-4.0 & 5.0-V-1 for further information.

Compound: PFOS

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.995 - 978	24.8 - 978	83-103	4.67-11.0
Low Curve	4.94 - 248	4.94 - 248	85-104	5.34-12.0
High curve	97.8 - 978	97.8 – 978	85-106	4.84-9.80
1/X	0.995 - 978	4.94 - 978	94-111	4.60-10.5

Compound: PFOSA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	4.93 - 976	88-103	5.10-14.7
Low Curve	4.93 - 97.6	4.93 - 97.6	87-105	9.85-14.7
High curve	24.8 - 976	24.8 - 978	93-102	5.08-13.9
1/X	0.993 - 976	4.93 - 976	94-103	5.10-14.5

Compound: PFOSAA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.991 - 974	24.7 - 974	81-111	4.18-10.6
Low Curve	4.92 - 247	9.74 - 247	97-107	6.38-21.8
High curve	49.2 - 974	97.4 - 974	85-108	4.33-12.5
1/X	0.991 - 974	9.74 - 974	95-115	4.11-23.2

Compound: EtFOSE-OH

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	49.3 - 976	77-110	11.2-25.5
Low Curve	4.93 – 97.6	9.76 – 97.6	97-107	14.1-21.3
High curve	49.3 - 976	97.6 - 976	90-109	11.5-19.6
1/X	0.993 - 493	9.76 - 976	86-111	11.1-21.2

Compound: PFOSEA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	24.8 - 976	96-106	10.1-16.2
Low Curve	4.93 - 248	9.76 - 248	91-110	11.8-19.5
High curve	49.3 - 976	49.3 - 976	86-106	10.2-18.2
1/X	0.993 - 976	9.76 - 976	95-117	10.1-19.1

Compound: M556

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	24.8 - 976	88-106	4.82-17.9
Low Curve	4.93 - 97.6	9.76 – 97.6	100-105	5.95-18.2
High curve	97.6 - 976	97.6 - 976	81-111	5.11-9.74
1/X	0.993 - 976	9.76 - 976	97-110	4.77-19.5

Ion Pair Standard Curves – Fluids

Prep date(s):
Analyte(s):
Sample matrix:

Standard number:
Equipment number:
Final solvent and TN:
Blank fluid/identifier:

Method/revision:
Target analyte(s):
FC mix std approx. 0.500 ppm:
FC mix std approx. 5.00 ppm:
FC mix std approx. 50.0 ppm:
Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix

PFOS Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	All Am't spiked mL	All Final vol mL
0.500	0.507	0.532	0.501	0.521	0.501	0.010	1.015
0.500	0.507	0.532	0.501	0.521	0.501	0.020	1.025
5.00	5.07	5.32	5.01	5.21	5.01	0.005	1.010
5.00	5.07	5.32	5.01	5.21	5.01	0.010	1.015
5.00	5.07	5.32	5.01	5.21	5.01	0.020	1.025
50.0	50.1	53.2	50.1	52.1	50.1	0.005	1.010
50.0	50.1	53.2	50.1	52.1	50.1	0.010	1.015
50.0	50.1	53.2	50.1	52.1	50.1	0.015	1.020
50.0	50.1	53.2	50.1	52.1	50.1	0.020	1.025

Calculated concentrations of standards in the sample matrix

PFOS Final conc ng/mL	PFOSA Final conc ng/mL	PFOSAA Final conc ng/mL	EtFOSE Final conc ng/mL	PFOSEA Final conc ng/mL	M556 Final conc ng/mL	Surrogate Std conc ng/mL	All Am't spiked mL
4.93	5.00	5.24	4.94	5.01	5.13	100	0.005
9.76	9.89	10.4	9.78	9.93	10.2		
24.8	25.1	26.3	24.8	25.2	25.8	**Surrogate**	
49.3	50.0	52.4	49.4	50.1	51.3	Final conc	
97.6	98.9	104	97.8	99.3	102	ng/mL	
248	251	263	248	252	258	500	
493	500	524	494	501	513		
735	746	782	737	749	766		
976	989	1038	978	993	1017		

Validated ranges – approximate concentrations

Serum	PFOS	PFOSA	PFOSAA	EtFOSE-OH	PFOSEA	M556
Rabbit	5.00-1000	5.00-1000	5.00-1000	5.00-1000	5.00-1000	5.00-1000
Bovine	Estimates only. Use values for rabbit.					
Rat	Estimates only. Use values for rabbit.					
Monkey & Plasma	Estimates only. Use values for rabbit.					
Human	Estimates only. Use values for rabbit.					

3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICAL COMPOUNDS FROM LIVER FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-6.0

Adoption Date: 07/22/99

Revision Date: NA

Author: Lisa Clemen, Robert Wynne

Approved By:

Laboratory Manager	Date 7/22/99
Group Leader	Date 7/14/99
Technical Reviewer Lisa A Clemen	Date 07/14/99

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from liver.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

1.3 Matrices: Rabbit, rat, bovine, and monkey livers or other tissues as designated in the validation report.

2.0 Summary of Method

2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver, or other tissues, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals can be extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard. An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL methanol then filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

2.2 These sample extracts are analyzed following method ETS-8-7.0 or other appropriate methods.

3.0 Definitions

3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3$

3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$

3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2$

3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$

3.5 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$

3.6 M556: $C_8F_{17}SO_2N(H)(CH_2COOH)$

3.7 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

4.0 Warnings and Cautions

4.1 **Health and Safety Warnings:**

4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

5.0 Interferences

5.1 There are no interferences known at this time.

6.0 Equipment

6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.

6.1.1 Ultra-Turrax T25 Grinder for grinding liver samples

6.1.2 Vortex mixer, VWR, Vortex Genie 2

6.1.3 Centrifuge, Mistral 1000 or IEC

6.1.4 Shaker, Eberbach or VWR

6.1.5 Nitrogen Evaporator, Organomation

6.1.6 Balance (sensitivity to 0.100 g)

7.0 Supplies and Materials

7.1 Gloves

7.2 Dissecting scalpels

7.3 Eppendorf or disposable pipettes

7.4 Nalgene bottles, capable of holding 250 mL and 1 L

7.5 Volumetric flasks, glass, type A

7.6 I-CHEM vials, 40 mL glass

7.7 Plastic sampule vials, Wheaton, 6 mL (or appropriate size)

7.8 Centrifuge tubes, polypropylene, 15 mL

7.9 Labels

7.10 Oxford Dispensor – 3.0 to 10.0 ml

7.11 Syringes, capable of measuring 5 µL to 50 µL

7.12 Graduated pipettes

7.13 Syringes, disposable plastic, 3 cc

7.14 Syringe filters, nylon, 0.2 µm, 25 mm

7.15 Timer

7.16 Crimp cap autovials and caps

7.17 Crimpers

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 Reagents and Standards

8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and be provided by a Milli-Q TOC Plus™ system

8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

8.4 Sodium carbonate (Na_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), J.T. Baker or equivalent

8.6 Methyl-*tert*-butyl ether, Omnisolv, glass distilled or HPLC grade

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Liver, frozen from supplier

8.9 Dry ice from supplier

8.10 **Fluorochemical standards**

8.10.1 PFOS (3M Specialty Chemical Division), molecular weight = 538

8.10.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

8.10.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585

8.10.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570

8.10.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527

8.10.6 M556 (3M Specialty Chemical Division), molecular weight = 557

8.10.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428

8.10.8 Other fluorochemicals, as appropriate

8.11 Reagent preparation

NOTE: When preparing larger volumes than listed in reagent, standard, or surrogate preparation, adjust accordingly.

8.11.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.11.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.

8.11.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.11.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.

8.11.4 0.25 M sodium carbonate/sodium bicarbonate buffer ($Na_2CO_3/NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.12 Standards preparation

8.12.1 Prepare PFOS standards for the standard curve.

8.12.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)

8.12.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.

8.12.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm (μg/mL).

8.12.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.12.6 Dilute the stock solution with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.12.7 Dilute the stock solution with methanol for a working standard 3 solution of approx. 0.50 ppm.

8.13 Surrogate stock standard preparation

8.13.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{13}SO_3H$ into a 50 ml volumetric flask and record the actual weight.

8.13.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.13.3 Prepare a surrogate working standard. Transfer approximately 1.0 ml of surrogate stock to a 10 ml volumetric flask and bring to volume with methanol for a working standard of 10-20 ppm. Record the actual volume transferred.

9.0 Sample Handling

9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

10.0 Quality Control

10.1 Matrix blanks and method blanks

10.1.1 An aliquot of 1.0 mL methanol is used as a solvent blank.

10.1.2 Extract two 1.0 mL aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.1.3 Extract two 1.0 mL aliquots of liver homogenate following this procedure and use as matrix blanks. Refer to **11.1.6**.

10.2 Matrix spikes

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually a control liver received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

10.3 Continuing calibration verifications

10.3.1 Prepare continuing calibration verification samples to ensure the accuracy of the initial calibration curve.

10.3.2 Prepare, at a minimum, one continuing calibration verification sample per group of 10 samples. For example, if a sample set = 34, four verifications are prepared and extracted.

10.3.3 Prepare each continuing calibration verification from the same matrix used to prepare the initial curve.

10.3.4 The expected concentrations will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

11.1.1 Weigh approximately 40 g of liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q™ water. Grind to a homogeneous solution.

11.1.2 If 40 g is not available, use appropriate amounts of liver and water to ensure a 1:5 ratio.

11.1.3 Refer to **13.0** to calculate the actual density of liver homogenate and the concentration of solid liver tissue dispersed in 1.0 mL of homogenate solution.

11.1.5 Add 1 mL of homogenate to a 15 mL centrifuge tube. Re-suspend solution by shaking between aliquots while preparing a total of eighteen 1 mL aliquots of homogeneous solution in 15 mL centrifuge tubes.

11.1.6 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks.

11.1.7 Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen samples, two matrix blanks, and two method blanks.

11.1.8 Refer to validation reports **ETS-8-6.0 and ETS-8-7.0-V-1** or **Attachment B**, which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.9 Use **Attachment C** as an aid in calculating the concentrations of the working standards. Refer to **13.0** to calculate actual concentrations of PFOS in calibration standards.

11.2 To each working standard, blank, or continuing verification, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb – 1000ppb.

11.3 Extract spiked liver homogenates following **12.14-12.25** of this method. Use these standards to establish each initial curve on the mass spectrometer.

Table 1
Approximate Spiking Amounts for Calibration Standards

Working Standard (Approx. Conc.)	µl	Approx. final conc. of PFOS in liver
-	-	Blank
0.50 ppm	2	0.005 ppm
0.50 ppm	4	0.010 ppm
0.50 ppm	10	0.025 ppm
0.50 ppm	20	0.050 ppm
0.50 ppm	40	0.100 ppm
5.0 ppm	10	0.250 ppm
5.0 ppm	20	0.500 ppm
5.0 ppm	30	0.750 ppm
50 ppm	4	1.00 ppm

12.0 PROCEDURE

12.1 Obtain frozen liver samples.

12.2 Cut approximately 1 g of liver using a dissecting scalpel. This part of the procedure is best performed quickly, not allowing the liver to thaw.

12.3 Weigh the sample directly into a tared plastic sampule vial.

12.4 Record the liver weight in the study notebook.

12.5 Return unused liver portions to freezer.

12.6 Add 2.5 mLs of water to sampule vial.

12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.

12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.

12.9 Take the grinder apart and clean it with methanol after each sample. Refer to AMDT-EP-22.

12.10 Cap the sample and vortex for 15 seconds. Label the sampule vial with the study number, weight, liver ID, date and analyst initials.

12.11 Pipette 1.0 mL, or other appropriate volume, of homogenate into a 15 mL polypropylene centrifuge tube. Label the centrifuge tube with the identical information as the sampule vial. Refer to attached worksheet for documenting the remaining steps.

12.12 Pipette two 1 mL aliquots of Milli-Q™ water to centrifuge tubes. These will serve as method blanks.

12.13 Spike all samples, including blanks and standards ready for extraction with surrogate standard as described in section **11.2**.

12.14 Spike each matirx with the appropriate amount of standard as described in 11.1, or Table 1 of that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.

12.15 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.

12.16 Check to ensure 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.

12.17 To each sample, add 1 mL 0.5 M TBA and 2 mL of the 0.25 M sodium carbonate/sodium bicarbonate buffer.

12.18 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.

12.19 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.

12.20 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.

12.21 Label a fresh 15 mL centrifuge tube with the same information as in **12.10**.

12.22 Remove 4.0 mL of the organic layer to the fresh 15 mL centrifuge tube.

12.23 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.

12.24 Add 1.0 mL to each centrifuge tube using a graduated pipette.

12.25 Vortex mix for 30 seconds.

12.26 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.

12.27 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.

12.28 Cap and store extracts at room temperature or at approximately 4 °C until analysis.

12.29 Complete the extraction worksheet, attached to this document, and tape in study notebook or include in study binder, as appropriate.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.1 Calculate the average density of the liver homogenate by recording each mass of ten separate 1.0 mL aliquots of homogenate.

$$\text{Average density (mg/mL)} = \frac{\text{Average mass (mg) of the aliquots}}{\text{1.0 mL aliquot}}$$

13.1.2 Calculate the amount of liver (mg) per 1.0 mL homogenate (or concentration of dispersed solid tissue per mL of homogenate suspension) using the following equation:

$$\frac{\text{g of Liver x Average density* of homogenate (mg/mL)}}{\text{(g of Liver + g of Water)}}$$

* refer to **13.1.1** for details.

13.1.3 Calculate actual concentrations of PFOS and other fluorochemicals in calibration standards using the following equation:

$$\frac{\mu\text{L of Standard x Concentration } (\mu\text{g /mL})}{\text{mg Liver / 1 mL homogenate*}} = \text{Final Concentration } (\mu\text{g/g or mg/kg}) \text{ of PFOS in Liver}$$

*refer to **13.1.2** for details.

14.0 METHOD PERFORMANCE

14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (refer to **Attachments B and C**).

14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks.

14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.

14.2.3 Continuing calibration verification samples to determine the continued accuracy of the initial calibration curve.

14.3 Refer to section 14 of **ETS-8-7.0** for method performance criteria.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 Records

16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in the 3-ring study binder, as appropriate.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard calculation and concentration worksheet

18.0 References

18.1 The validation report associated with this method is **ETS-8-6.0 & 7.0-V-1.**

18.2 AMDT-EP-22, "Routine Maintenance of Ultra-Turrax T-25"

18.3 FACT-M-1.1, "Extraction of PFOS or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

19.0 Affected Documents

19.1 ETS-8-7.0, "Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 Revisions

Revision Number.	Reason For Revision	Revision Date

Study # ____ Matrix ____ Box # ____ Wk/Day ____ Date Spiked/Analyst	Surrogate Std approx. ppm actual ppm # ____	FC Mix Std approx. 0.5 ppm actual ppm # ____	FC Mix Std approx. 5 ppm actual ppm # ____	FC Mix Std approx. 50 ppm actual ppm # ____	Comments
CCV					
MS					
MSD					
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

Blank Liver Homogenate: Std #	Liver amount = g	
Liver Extraction Method :		**Date & Initials**
Spike surrogate and Standard mix, Vortex 15 sec.		
Pipette 1 mL of Liver Solution		
Pipette 1 mL of †0.5 M TBA, pH 10. pH = ____	Std. # ____	
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer	Std. # ____	
Dispense 5ml of Methyl-t-Butyl Ether	TN-A-____	
Shake 20 min.	Shaker Speed	
Centrifuge 20-25 min.	Centrifuge Speed	
Remove a 4 mL aliquot of organic layer		
Put on Nitrogen Evaporator to dryness	Evaporator Temperature	
Add 1.0 mL of Methanol	TN-A- ____	
Vortex 30 sec.		
Filter using a 3cc B-D syringe with a 0.2µm SRI filter into autosample vial		

Cont. Cal. Verifications used the same matrix as for the standard curve.

MDL/LOQ values for rabbit liver

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	8.45	26.9	30 ppb – 1200 ppb
PFOSA	3.50	11.1	12 ppb – 1200 ppb
PFOSAA	24.6	78.3	30 ppb – 1200 ppb
EtFOSE-OH	108	345	60 ppb – 900 ppb*
M556	82.3	262	60 ppb – 1200 ppb
PFOSEA	33.9	108	30 ppb- 1200 ppb

MDL/LOQ values in rat, bovine, and monkey liver were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the rabbit liver curves to determine equivalence. Responses in the rat, bovine, and monkey liver curves were equivalent to the rabbit responses, therefore, their MDL and LOQ will be assumed to be equivalent to those values as determined for the rabbit liver.

Refer to LOQ Summary and MDL study in ETS-8-6.0 & 7.0-V-1 for further information
* EtFOSE-OH estimates only for MDL and LOQ. Did not meet criteria for validation.

Compound: PFOS

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.19 - 1237	12 - 1200	12 - 1200	6 - 300	12 - 300	60 - 1200	60 - 1200

Compound: PFOSA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.19 - 1237	12 - 1200	12 - 1200	12 - 300	12 - 300	60 - 1200	60 - 1200

Compound: PFOSAA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.16 - 1232	12 - 1200	30 - 1200	30 - 900	60 - 900	N/A	N/A

Compound: EtFOSE-OH

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 900	31 - 900	N/A	N/A	N/A	N/A

Compound: PFOSEA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 1200	31 - 1200	N/A	N/A	N/A	N/A

Compound: M556

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 1200	60 - 1200	N/A	N/A	N/A	N/A

Ion Pair Standard Curves – Tissue

Prep date(s):
Analyte(s):
Sample matrix:

Standard number:
Equipment number:
Final solvent and TN:
Blank liver/identifier:

Method/revision:
Target analyte(s):
FC mix std approx. 0.500 ppm:
FC mix std approx. 5.00 ppm:
FC mix std approx. 50.0 ppm:
Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix

PFOS Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	Std conc ug/mL	All Am't spiked mL	All Density g
0.500	0.500	0.500	0.500	0.500	0.500		0.002	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.004	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.010	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.020	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.040	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.010	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.020	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.030	0.167
50.0	50.0	50.0	50.0	50.0	50.0		0.004	0.167

Calculated concentrations of standards in the sample matrix

PFOS Final conc ng/g	PFOSA Final conc ng/g	PFOSAA Final conc ng/g	EtFOSE Final conc ng/g	PFOSEA Final conc ng/g	M556 Final conc ng/g	Std conc ng/g	Surrogate Std conc ng/mL	All Am't spiked mL
5.99	5.99	5.99	5.99	5.99	5.99		100	0.005
12.0	12.0	12.0	12.0	12.0	12.0			
29.9	29.9	29.9	29.9	29.9	29.9		**Surrogate**	
59.9	59.9	59.9	59.9	59.9	59.9		Final conc	
120	120	120	120	120	120		ng/mL	
299	299	299	299	299	299		0.500	
599	599	599	599	599	599			
898	898	898	898	898	898			
1198	1198	1198	1198	1198	1198			

Validated ranges – approximate concentrations

Liver	PFOS	PFOSA	PFOSAA	EtFOSE-OH	POAA	PFOSEA
Rabbit	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb
Bovine	Estimates only, use rabbit values.					
Rat	Estimates only, use rabbit values.					
Monkey	Estimates only, use rabbit values.					

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF FLUOROCHEMICALS IN LIVER EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: FACT-M-2.0

Adoption Date: 5/26/98

Revision Date: N/A

Author: Lisa Clemen

Approved By:

[signature] 5/26/98
Laboratory Manager — Date

[signature] 5/26/98
Group Leader — Date

Lisa A Clemen 5/27/98
Technical Reviewer — Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the analysis of extracts of liver or other tissues for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Potassium perfluorooctanesulfonate, anionic fluorochemical surfactants, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, bovine, and monkey livers or other livers as designated in the validation report.

2.0 SUMMARY OF METHOD

2.1 This method describes the analysis of fluorochemical surfactants extracted from liver using HPLC-electrospray/mass spectrometry. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the potassium perfluorooctanesulfonate (PFOS) anion, M/Z= 499. Samples may also be screened to verify compound identification.

3.0 DEFINITIONS

3.1 None.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

4.1.1 Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

4.2 Cautions:

4.2.1 Do not run solvent pumps above capacity of 400 bar (5800 psi). If pressure goes over 400 bar, the HP1100 will initiate automatic shutdown.

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

5.1 Teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

6.1 Equipment listed below may be changed in order to optimize the system.

6.1.1 Micromass Electrospray Mass Spectrometer

6.1.2 HP1100 low pulse solvent pumping system and autosampler.

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 Nitrogen gas, refrigerated liquid, regulated to approximately 100 psi.

7.1.2 HPLC column, specifics to be determined by the analyst.

7.1.3 Capped autovials or capped 15 mL centrifuge tubes.

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent.

8.1.2 Milli-Q™ water, all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus system.

8.1.3 Ammonium acetate, HPLC grade or equivalent.

8.2 Standards

8.2.1 Typically one H_2O blank, one liver blank, and seven liver standards are prepared during the extraction procedure. See FACT-M-1.

9.0 Sample Handling

9.1 Fresh liver standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.

9.2 If analysis will be delayed, extracted standards and samples may be refrigerated until analysis can be performed.

10.0 Quality Control

10.1 Matrix Blanks and Method Blanks

10.1.1 Analyze a method blank and matrix blank prior to each calibration curve.

10.2 Matrix Spikes

10.2.1 Analyze a matrix spike and matrix spike duplicate with each analysis.

10.2.2 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.2.3 See section 13 to calculate percent recovery.

10.3 Continuing Calibration Checks

10.3.1 Analyze a mid-range calibration standard after every tenth sample. If a significant change (± 30%) in peak area occurs, relative to the initial standard curve, stop the run. Only those samples analyzed before the last acceptable calibration standard will be used. The remaining samples must be reanalyzed.

10.3.2 See section 13 to calculate percent difference.

10.4 System Suitability

10.4.1 System suitability (e.g. peak area, retention time and peak shape, etc.) will be assessed for each run.

11.0 Calibration and Standardization

11.1 Analyze the extracted liver standards prior to and following each set of extracts. The mean of two standard values, at each standard concentration, will be plotted by linear regression for the calibration curve using MassLynx or other suitable software.

11.2 The r^2 value for the data should be 0.98 or greater. Lower values may be acceptable at the discretion of the analyst.

11.3 If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using letter-MO-DAY-last digit of year-sample number, assign a method (MS) for acquiring, and type in sample descriptions.

12.1.2 To create a method click on scan button in the Acquisition control panel and select SIR. Set Ionization Mode as appropriate and mass to 499 or other appropriate masses.. A scan is usually collected along with the SIRs. Save method.

12.1.3 Typically the sample list begins with the first set of liver standards and ends with the second set of standards.

12.1.4 Samples are analyzed with a continuing calibration check injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

12.2.1 Set up sample tray according to the sample list prepared in section 12.1.1.

12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

12.2.2.1 Sample size = 10 µL injection with a sample wash

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 15 minutes

12.2.2.4 Solvent ramp =

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	45%	55%
7.5 min.	90%	10%
11.0 min.	90%	10%
11.5 min.	45%	55%

Note: In this instrument configuration, the run must be set up on the electrospray software with a "Waiting for inlet start" message before the "Start" button is pressed on the HP Workstation.

12.2.2.5 Press the "Start" button.

12.3 Instrument Sep-up

12.3.1 Refer to AMDT-EP-31 for more details.

12.3.2 Check the solvent level in reservoirs and refill if necessary.

12.3.3 Check the stainless steel capillary at the end of the probe. Use an eye piece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.

12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe.

12.3.6 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

12.3.6.3 LC constant flow mode flow rate 10 – 500 uL/min

12.3.6.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the instrument is operating correctly.)

12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.

12.3.8 Record tune parameters in the instrument log.

12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.

12.3.10 Click on start button in the Acquisition Control Panel. Press the start button at top of sample list. Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.1 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result - Background Result}}{\text{Expected Result}} \times 100$$

13.1.2 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc. - Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

13.1.3 Calculate actual concentration of PFOS anion in total liver (mg):

$$\frac{\left(\dfrac{\text{ug PFOS anion calc. from std curve}}{\text{g of liver used for analysis}}\right)}{1000\ \text{ug} / 1\ \text{mg}} \times \text{Total mass of liver (g)}$$

14.0 Method Performance

14.1 The method detection limit is equal to at least three times the baseline noise in the matrix blank.

14.2 The practical quantitation limit is equal to the lowest standard in the calibration curve.

15.0 Pollution Prevention and Waste Management

15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers. All containers are located in the laboratory.

16.0 Records

16.1 Store chromatograms in the study folder. Each chromatogram should have the following information included either in the header or hand written on the chromatogram: study number, sample name, extraction date, and dilution factor (if applicable).

16.2 Plot calibration curve by linear regression and store in the study folder.

16.3 Print sample list from MassLynx and tape into the instrument runlog.

16.4 Print data integration summary from MassLynx and tape into the instrument runlog.

16.5 Copy instrument runlog pages, including instrument parameters and sample results, and tape into appropriate study notebook.

16.6 Summarize data using suitable software and store in the study folder.

16.7 Back up electronic data to appropriate media. Record in study notebook the file name and location of backup electronic data.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment A: FACT-M-2 Data reporting spreadsheet

17.2 The validation report associated with this method is FACT-M-1.0 & 2.0-V-1.

18.0 References

18.1 AMDT-EP-31, "Operation of VG Platform Electrospray Mass Spectrometer"

19.0 AFFECTED DOCUMENTS

19.1 FACT-M-1.0, "Extraction of Potassium Perfluorooctanesulfonate from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date

Laboratory Study

Study:
Test Material:
Matrix/Final Solvent:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Filename:
R-Squared Value:
Slope:
Y Intercept:
Date of Extraction/Analyst:
Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.
Group/Dose: Taken from the study folder.
Sample#: Taken from the study folder.
Concentration (ug/mL): Taken from the MassLynx integration summary.
Initial Volume (mL): Taken from the study folder.
Dilution Factor: Taken from the study folder.
Final Conc. (ug/mL): Calculated by dividing the initial volume from the concentration

Working Copy

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICALS IN SERUM OR OTHER FLUID EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: FACT-M-4.1

Adoption Date: 4/22/98

Revision Date: 10-1-98

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager — Date: 10/1/98

Group Leader — Date: 9/29/98

Technical Reviewer — Lisa A Clemen — Date: 9/29/98

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the analysis of extracts from serum or blood for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, bovine, or monkey serum and rat whole blood or milk curd.

2.0 Summary of Method

2.1 This method describes the analysis of fluorochemical surfactants extracted from serum, whole blood, or milk curd using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the potassium perfluorooctanesulfonate (PFOS) anion, M/Z= 499. Samples may also be analyzed using an API/MS/MS system to further verify compound identification.

3.0 Definitions

3.1 Atmospheric Pressure Ionization (API): The Micromass platform systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APcI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e. not under a vacuum).

3.2 Electrospray Ionization (ES, ESI): a method of ionization performed at atmospheric pressure, whereby ionization occurs through the production of tiny charged droplets in a strong electrical field.

3.3 Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS): The API platforms are equipped with quadrupole mass selective detectors. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or a series (MS/MS) for more specific fragmentation information.

3.4 Conventional vs. Z-spray probe interface: The latest models of Micromass platform systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e. Z-spray components are compatible with other Z-spray systems, etc.)

3.5 Mass Lynx Software: System software designed for the specific operation of these platform systems. Currently MassLynx has Windows 95 and WindowsNT 3.1 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Platform II or Quattro II MassLynx or MassLynx NT USER'S GUIDE).

4.0 Warnings and Cautions

4.1 Health and Safety Warnings:

4.1.1 Use caution with the voltage cables for the probe. The probe employs a voltage of approximately 5000 Volts.

4.1.2 When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

4.2 Cautions:

4.2.1 Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

5.1 To minimize interferences when analyzing samples for perfluorooctanoate(POAA), teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

6.1 Equipment listed below may be modified in order to optimize the system.

6.1.1 Micromass Electrospray Mass Spectrometer

6.1.2 HP1100 low pulse solvent pumping system and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi

7.1.2 HPLC analytical column, specifics to be determined by the analyst

7.1.3 Capped autovials or capped 15 ml centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

8.1.2 Milli-Q™ water, all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus system

8.1.3 Ammonium acetate, reagent grade or equivalent

8.2 Standards

8.2.1 Typically one method blank, one matrix blank, and ten matrix standards are prepared during the extraction procedure. See **FACT-M-3.1.**

9.0 SAMPLE HANDLING

9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 ml centrifuge tubes until analysis.

9.2 If analysis will be delayed, extracted standards and samples can be refrigerated at approximately 4° C until analysis can be performed.

10.0 QUALITY CONTROL

10.1 Method Blanks and Matrix Blanks

10.1.1 Analyze a method blank and a matrix blank prior to each calibration curve.

10.2 Matrix Spikes

10.2.1 Analyze a matrix spike and matrix spike duplicate per forty samples. With a minimum of 2 spikes per batch.

10.2.2 Expected spike concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.2.3 See Section 13 to calculate percent recovery.

10.3 Continuing Calibration Checks

10.3.1 Analyze a mid-range calibration standard after every tenth sample. If a significant change (± 30%) in peak area occurs, relative to the initial standard curve, stop the run. Only those samples analyzed before the last acceptable calibration standard will be used. The remaining samples must be reanalyzed.

10.3.2 See Section 13 to calculate percent difference.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Analyze the extracted matrix standards prior to and following each set of extracts. The mean of two standard values, at each standard concentration, will be plotted by linear regression (r^2)for the calibration curve using MassLynx or other suitable software.

11.2 The r^2 value for the data should be 0.980 or greater. Lower values may be acceptable at the discretion of the analyst and documented approval of the Project Lead.

11.3 If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

11.4 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using letter-MO-DAY-last digit of year-sample number, assign a method (MS) for acquiring, and type in sample descriptions.

12.1.2 To create a method click on scan button in the Acquisition control panel and select SIR (Single Ion Recording). Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. See Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM (Multiple Reaction Monitoring).

12.1.3 Typically the analytical batch run sequence begins with a set of extracted matrix standards and ends with a set of extracted matrix standards.

12.1.4 Samples are analyzed with a continuing calibration check injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

12.2.1 Set up sample tray according to the sample list prepared in Section **12.1.1**.

12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

12.2.2.1 Sample size = 10 µL injection with a sample wash

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 15 minutes

12.2.2.4 Solvent ramp =

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	45%	55%
7.5 min.	90%	10%
11.0 min.	90%	10%
11.5 min.	45%	55%

Note: In this instrument configuration, the run must be set up on the electrospray software with a "Waiting for inlet start" message before the "Start" button is pressed on the HP Workstation.

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

12.3.1 Refer to **FACT-EP-3.0** for more details.

12.3.2 Check the solvent level in reservoirs and refill if necessary.

12.3.3 Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.

12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe.

12.3.6 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

12.3.6.3 HPLC constant flow mode flow rate 10 – 500 μL/min

12.3.6.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)

12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.

12.3.8 Record tune parameters in the instrument log.

12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.

12.3.10 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, see appropriate MassLynx USER'S GUIDE). Press the start button at top of sample list. Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result - Background Result}}{\text{Expected Result}} \times 100$$

13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc. - Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

13.1.6 Calculate actual concentration of PFOS, or other fluorochemical, in matrix (μg/ml):

$$\frac{\dfrac{\text{(ng of PFOS calc. from std. Curve x Dilution Factor)}}{\text{(Initial Volume of matrix (ml) + ml of Surrogate Standard)}}}{\text{Final Volume (mL)}} \times \frac{1\ \mu\text{g}}{1000\ \text{ng}}$$

14.0 METHOD PERFORMANCE

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see **FACT-M-3.1, Attachment A** for a listing of current validated MDL and LOQ values.

14.2 Method Blanks and Matrix Blanks

14.2.1 Method blanks and matrix blanks will be analyzed with each sample set for possible contamination or carryover. Values are expected to fall below the lowest standard in the calibration curve.

14.3 Matrix Spikes

14.3.1 Matrix spikes are analyzed with each sample set and the percent recoveries are expected to fall within ± 30% of the spiked concentration.

14.4 Continuing Calibration Checks

14.4.1 Continuing calibration checks are analyzed at a minimum of after every 10 samples with each sample set. The percent recoveries are expected to fall within ± 30% of the spiked concentration.

14.5 If any criteria listed in the method performance section isn't met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. All actions will be documented in the instrument runlog, the maintenance log, or on the summary sheet with the sample results.

15.0 Pollution Prevention and Waste Management

15.1 Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 Records

16.1 Store chromatograms in the study or project folder. Each chromatogram must have the following information included either in the header or hand written on the chromatogram: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.

16.2 Plot calibration curve by linear regression and store in the study folder.

16.3 Print sample list from MassLynx and tape into the instrument runlog.

16.4 Print data integration summary from MassLynx and tape into the instrument runlog.

16.5 Copy instrument runlog pages, including instrument parameters and sample results, and store in appropriate study folder.

16.6 Summarize data using suitable software and store in the study folder.

16.7 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment A: FACT-M-4.1 Data reporting spreadsheet

17.2 The validation report associated with this method is **FACT-M-3.1 & 4.1-V-1**.

18.0 References

18.1 FACT-EP-3.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Platform Systems"

19.0 Affected Documents

19.1 FACT-M-3.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 Revisions

Revision Number.	Reason For Revision	Revision Date
1	*Validation of method to include 7 fluorochemicals addition of whole blood matrix, surrogate standard, new API/MS(MS) systems, monkey sera cross validation, MDL study, updates in record keeping and storing policies, etc.*	07/01/98

Attachment A

Laboratory Study

Study:
Test Material:
Matrix/Final Solvent:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Filename:
R-Squared Value:
Slope:
Y Intercept:
Date of Extraction/Analyst:
Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.
Group/Dose: Taken from the study folder.
Sample#: Taken from the study folder.
Concentration (ug/mL): Taken from the MassLynx integration summary.
Initial Volume (mL): Taken from the study folder.
Dilution Factor: Taken from the study folder.
Final Conc. (ug/mL): Calculated by dividing the initial volume from the concentration

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICALS IN SERUM EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-5.1

Adoption Date: 03/01/99

Revision Date: 4/26/99

Author: Lisa Clemen, Robert Wynne

Approved By:

Laboratory Manager	Date 4/26
Kirsten H. — Group Leader	Date 4/26/99
Lisa A Clemen — Technical Reviewer	Date 04/26/99

1.0 SCOPE AND APPLICATION

1.1 Scope: This method describes the analysis of serum extracts for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, bovine, monkey, and human serum, or other fluids as designated in the validation report.

2.0 SUMMARY OF METHOD

2.1 This method describes the analysis of fluorochemical surfactants extracted from serum or other fluids, using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion, m/z= 499. Additionally, samples may be analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the parent ion.

3.0 DEFINITIONS

3.1 Atmospheric Pressure Ionization (API): The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APcI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e., not under a vacuum).

3.2 Electrospray Ionization (ES, ESI): a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.

3.3 Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS): The API Quattro II triple quadrupole systems are equipped with quadrupole mass selective detectors. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or a series (MS/MS) for more specific fragmentation information.

3.4 Conventional vs. Z-spray probe interface: The latest models of Micromass Quattro II triple quadrupole systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e., Z-spray components are compatible with some other Z-spray systems, etc.)

3.5 Mass Lynx Software: System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

4.1.1 Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 Volts.

4.1.2 When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

4.2 Cautions:

4.2.1 Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

5.1 To minimize interferences when analyzing samples, teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.

6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source

6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi (House air system)

7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.

7.1.3 Capped autovials or capped 15 mL centrifuge tubes.

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

8.1.2 Milli-Q™ water, all water used in this method should be Milli-Q™ water or equivalent, and may be provided by a Milli-Q TOC Plus system or other vendor

8.1.3 Ammonium acetate, reagent grade or equivalent

8.2 Standards

8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. See **ETS-8-4.1.**

9.0 SAMPLE HANDLING

9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.

9.2 If analysis will be delayed, extracted standards and samples can be refrigerated at approximately 4° C, or at room temperature, until analysis can be performed.

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method Blanks and Matrix Blanks

10.1.1 Solvent blanks, method blanks and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.

10.1.2 Analyze a method blank and a matrix blank prior to each calibration curve.

10.2 Matrix Spikes

10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.

10.2.3 Analyze a matrix spike and matrix spike duplicate per forty samples, with a minimum of 2 spikes per batch.

10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Verifications

10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Analyze the extracted matrix standards prior to and following each set of extracts. The average of two standard curves will be plotted by linear regression ($y = my + b$), weighted $1/x$, not forced through zero, using MassLynx or other suitable software.

11.2 If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

11.3 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using MO-DAY-last digit of year-sample number, assign a method (MS) for acquiring, and type in sample descriptions.

12.1.2 To create a method click on scan button in the Acquisition control panel and select SIR (Single Ion Recording) or MRM. Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. See Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM (Multiple Reaction Monitoring).

12.1.3 Typically the analytical batch run sequence begins with a set of extracted matrix standards and ends with a set of extracted matrix standards.

12.1.4 Samples are analyzed with a continuing calibration check injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

12.2.1 Set up sample tray according to the sample list prepared in Section **12.1.1.**

12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

12.2.2.1 Sample size = 10 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 13.5 minutes

12.2.2.4 Solvent ramp =

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
8.50 min.	90%	10%
11.0 min.	90%	10%
12.0 min.	40%	60%

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

12.3.1 Refer to **ETS-9-24.0** for more details.

12.3.2 Check the solvent level in reservoirs and refill if necessary.

12.3.3 Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.

12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed.

12.3.6 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

12.3.6.3 HPLC constant flow mode, flow rate 10 – 500 μL/min

12.3.6.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)

12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.

12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.

12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.

12.3.10 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, see appropriate MassLynx USER'S GUIDE). Press the start button. Ensure start and end sample number includes all samples to be analyzed.

13.0 Data Analysis and Calculations

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result - Background Result}}{\text{Expected Result}} \times 100$$

13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc. - Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

13.1.6 Calculate actual concentration of PFOS, or other fluorochemical, in matrix (μg/mL):

$$\frac{\text{(ng of PFOS calc. from std. Curve x Dilution Factor)}}{\dfrac{\text{(Initial Volume of matrix (mL) + mL of Surrogate Standard)}}{\text{Final Volume (mL)}}} \times \frac{1\ \mu g}{1000\ ng}$$

14.0 METHOD PERFORMANCE

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see **ETS-8-4.1, Attachment B,** for a listing of current validated MDL and LOQ values.

14.2 Solvent Blanks, Method Blanks, and Matrix Blanks

14.2.1 Solvent blanks, method blanks, and matrix blanks values are must be below the lowest standard in the calibration curve

14.3 Calibration Curves

14.3.1 The r^2 value for the calibration curve must be 0.980 or better.

14.4 Matrix Spikes

14.4.1 Matrix spike percent recoveries are must be within ± 30% of the spiked concentration.

14.5 Continuing Calibration Verifications

14.5.1 Continuing calibration verification percent recoveries must be ± 30% of the spiked concentration.

14.6 If criteria listed in this method performance section isn't met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.

14.7 If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1 Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

16.1 Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.

16.2 Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.

16.3 Plot the calibration curve by linear regression, weighted 1/x, then print these graphs and store in the study folder.

16.4 Print data integration summary, integration method, and chromatograms, from MassLynx, and store in the study folder.

16.5 Summarize data using suitable software (Excel 5.0) and store in the study folder, see **Attachment A** for an example of a summary spreadsheet.

16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment A: ETS-8-5.1 Data summary spreadsheet.

18.0 References

18.1 FACT-M-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"

18.3 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1.**

19.0 Affected Documents

19.1 ETS-8-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 Revisions

Revision Number.	Reason For Revision	Revision Date
1	Section 6.1.2 Clarification of HP1100 system components. Section 11.1 Average of two curves, not standard values, are used for plotting linear regression and added the 1/x weighting of the curve. Section 12.2.2.4 Clarification of solvent ramp. Section 17.1 Changed from attachment B to A.	04/02/99

Laboratory Study

Study:
Test Material:
Matrix/Final Solvent:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Filename:
R-Squared Value:
Slope:
Y Intercept:
Date of Extraction/Analyst:
Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.
Group/Dose: Taken from the study folder.
Sample#: Taken from the study folder.
Concentration (ug/mL): Taken from the MassLynx integration summary.
Initial Volume (mL): Taken from the study folder.
Dilution Factor: Taken from the study folder.
Final Conc. (ug/mL): Calculated by dividing the initial volume from the concentration

Study #: FACT-TOX-098

3M Environmental Lab -- Method Modification

Method:

ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Sera Extracts Using HPLC-Electrospray/Mass Spectrometry"

Section modified: 10.3.2, 14.5.1, add sections 14.3.2-14.3.6

Effective date of modifications: April 26, 1999

Section 10.3.2

Method reads:

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

Modify method to read:

10.3.2 Analyze a mid-range calibration standard **at least** after every ten samples, with a minimum of one per batch.

Section 14.5.1

Method reads:

14.5.1 Continuing calibration verification percent recoveries must be within ± 30% of the spiked concentration.

Modify method to read:

14.5.1 At least one continuing calibration verification per ten samples must show a percent recovery within +/-30% of the spiked concentration.

Section 14.3.2

Method reads:

NA

Modify method to read:

14.3.2 The second (bracketing) calibration curve may be deactivated if instrumental drift affects the data. The first curve and acceptable calibration checks shall bracket usable data.

BACK TO MAIN

Study #: FACT-TOX-098

Section 14.3.3
Method reads:

NA

Modify method to read:

14.3.3 Calibration standards with peak areas less than 2 times the curve matrix blank should be deactivated to disqualify a data range that may be affected by background levels of the analyte.

Section 14.3.4
Method reads:

NA

Modify method to read:

14.3.4 Low or high curve points may be deactivated to optimize a linear range appropriate to the data.

Section 14.3.5
Method reads:

NA

Modify method to read:

14.3.5 A curve point may be deactivated if it deviates more than 30% from the theoretical value when the curve is evaluated over a linear range appropriate to the data.

Section 14.3.6
Method reads:

NA

Modify method to read:

14.3.6 A valid calibration curve must contain at least 5 active points.

Kristen Hansen 11/19/00
Signature of PAI and date

John L. Butenhoff 13 DEC 2000
Signature of Sponsor and date

Marvin T Case 13 Dec 2000
Signature of Study Director and date

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICALS IN LIVER EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-7.0

Adoption Date: 07/22/99

Revision Date: NA

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager — Date: 7/22/99

Group Leader — Date: 7/14/99

Technical Reviewer — Date: 07/14/99

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the analysis of liver extracts for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, bovine, monkey liver, or other tissues as designated in the validation report.

2.0 SUMMARY OF METHOD

2.1 This method describes the analysis of fluorochemical surfactants extracted from liver using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion, m/z = 499. Additionally, samples may be analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the selected parent ion.

3.0 DEFINITIONS

3.1 **Atmospheric Pressure Ionization (API):** The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APcI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e. not under a vacuum).

3.2 **Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.

3.3 **Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole mass spectrometer is equipped with two quadrupole mass selective detectors and a collision cell. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or an ion may be selected in the first quadrupole, fragmented in the collision cell, and these fragments may be analyzed in the second quadrupole.

3.4 **Conventional vs. Z-spray probe interface:** The latest models of Micromass Quattro II triple quadrupole (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e. Z-spray components are compatible with other Z-spray systems, etc.)

3.5 **Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details refer to the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

4.1.1 Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 Volts.

4.1.2 When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

4.2 Cautions:

4.2.1 Operate the solvent pumps below a back pressure of 400 bar (5800 psi). If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

5.1 To minimize interferences when analyzing samples, Teflon shall not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.

6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source.

6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 High purity grade air regulated to approximately 100 psi (house air system)

7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data

7.1.3 Capped autovials or capped 15 ml centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

8.1.2 Milli-Q™ water (ASTM type I), all water used in this method should be ATSM type I, or equivalent, and be provided by a Milli-Q TOC Plus system or other vendor

8.1.3 Ammonium acetate, reagent grade or equivalent

8.1.3.1 When preparing different amounts than those listed, adjust accordingly.

8.1.3.2 2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing 2000 mL Milli-Q™ water, mix until all solids are dissolved. Store at room temperature.

8.2 Standards

8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. Refer to **ETS-8-6.0.**

9.0 SAMPLE HANDLING

9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 ml centrifuge tubes until analysis.

9.2 If analysis will be delayed, extracted standards and samples may be stored at room temperature, or refrigerated at approximately 4° C, until analysis can be performed.

10.0 QUALITY CONTROL

10.1 Method Blanks and Matrix Blanks

10.1.1 Solvent blanks, method blanks, and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.

10.1.2 Analyze a method blank and a matrix blank prior to each calibration curve.

10.2 Matrix Spikes

10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.

10.2.3 Analyze a matrix spike and matrix spike duplicate per forty samples. With a minimum of 2 spikes per batch.

10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Checks

10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.

10.3.2 Analyze a mid-range calibration standard every tenth sample, with a minimum of one per batch.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Analyze the extracted matrix standards prior to and following each set of sample extracts. The average of two standard curves will be plotted by linear regression ($y = mx + b$), weighted $1/x$, not forced through the origin, using MassLynx or other suitable software.

11.2 If the curve does not meet requirements perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

11.3 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Set up the sample list.

12.1.1.1 Assign a sample list filename using MO-DAY-last digit of year-increasing letter of the alphabet starting with a

12.1.1.2 Assign a method (MS file) for acquiring

12.1.1.3 Assign an HPLC program (Inlet file)

12.1.1.4 Type in sample descriptions and vial position numbers

12.1.2 To create a method click on method in the Acquisition control panel then mass spectrometer headings and select SIR (Single Ion Recording) or MRM (Multiple Reaction Monitoring). Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. Refer to Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM.

12.1.3 Typically the analytical batch run sequence begins and ends with a set of extracted matrix standards.

12.1.4 Samples are analyzed with a continuing calibration verification injected standard after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

12.2.1 Set up sample tray according to the sample list prepared in Section **12.1.1**.

12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

12.2.2.1 Sample size = 10 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 9 minutes

12.2.2.4 Solvent ramp conditions

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
1.0 min.	40%	60%
4.5 min.	95%	5%
6.5 min.	95%	5%
7.0 min.	40%	60%
9.0 mi.	40%	60%

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

12.3.1 Refer to **ETS-9-24.0,** "Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source," for more details.

12.3.2 Check the solvent level in reservoirs and refill if necessary.

12.3.3 Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

12.3.4 Turn on the nitrogen.

12.3.5 Open the tune page. Clicks on operate to initiate source block and desolvation heaters.

12.3.6 Open the Inlet Editor.

12.3.6.1 Set HPLC pump to "On"

12.3.6.2 Set the flow to 10 - 500 uL/min or as appropriate

12.3.6.3 Observe droplets coming out of the tip of the probe. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed

12.3.6.4 Allow to equilibrate for approximately 10 minutes.

12.3.7 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:

12.3.7.1 Drying gas 250-400 liters/hour

12.3.7.2 ESI nebulizing gas 10-15 liters/hour

12.3.7.3 HPLC constant flow mode flow rate 10 – 500 μL/min

12.3.7.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)

12.3.7.5 Source block temperature 150°

12.3.7.6 Desolvation temperature 250°

12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.

12.3.9 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, refer to appropriate MassLynx User's Guide). Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Background Result}}{\text{Expected Result}} \times 100$$

13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

13.1.6 Calculate actual concentrations in matrix (µg/g):

$$\frac{(\text{ng of PFOS calc. from std. Curve} \times \text{Dilution Factor})}{\dfrac{(\text{Initial Weight of Liver (g)}}{\text{Final Volume (mL)}}} \times \frac{1\ \mu g}{1000\ ng}$$

14.0 METHOD PERFORMANCE

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Refer to **ETS-8-6.0, Attachment B** for a listing of current validated MDL and LOQ values.

14.2 Solvent Blanks, Method Blanks and Matrix Blanks

14.2.1 Solvent blanks, method blanks, and matrix blanks must be below the lowest standard in the calibration curve.

14.3 Calibration Curves

14.3.1 The r^2 value for the calibration must be 0.980 or better.

14.4 Matrix Spikes

14.4.1 Matrix spike percent recoveries must be within ± 30% of the spiked concentration.

14.5 Continuing Calibration Verification

14.5.1 Continuing calibration verification percent recoveries must be within ± 30% of the spiked concentration.

14.6 If criteria listed in the method performance section are not met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.

14.7 If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

15.0 Pollution Prevention and Waste Management

15.1 Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 Records

16.1 Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.

16.2 Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.

16.3 Plot the calibration curve by linear regression, weighted 1/x, then print these graphs and store in the study folder.

16.4 Print data integration summary, integration method, and chromatograms from MassLynx and store in the study folder.

16.5 Summarize data using suitable software (Excel 5.0+) and store in the study folder, refer to **Attachment A** for an example of a summary spreadsheet.

16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment A: ETS-8-7.0 Data summary spreadsheet

18.0 References

18.1 FACT-M-2.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"

18.3 The validation report associated with this method is **ETS-8-6.0 & 7.0-V-1**

19.0 Affected Documents

19.1 ETS-8-6.0, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver or Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

Revision Number	Reason For Revision	Revision Date

Laboratory Study

Study:
Test Material:
Matrix/Final Solvent:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Filename:
R-Squared Value:
Slope:
Y Intercept:
Date of Extraction/Analyst:
Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ng/g	Initial Wt. g	Dilution Factor	Final Conc. ug/g

Slope: Taken from linear regression equation.
Group/Dose: Taken from the study folder.
Sample#: Taken from the study folder.
Concentration (ng/g): Taken from the MassLynx integration summary.
Initial Wt. (g): Taken from the study folder.
Dilution Factor: Taken from the study folder.
Final Conc. (ug/g): Calculated by dividing the initial volume from the concentration

Study #: FACT-TOX-098

3M Environmental Lab -- Method Modification

Method:

ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

Section modified:	10.3.2, 14.5.1, add sections 14.3.2-14.3.6
Effective date of modifications:	July 22, 1999

Section 10.3.2

Method reads:

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

Modify method to read:

10.3.2 Analyze a mid-range calibration standard **at least** after every ten samples, with a minimum of one per batch.

Section 14.5.1

Method reads:

14.5.1 Continuing calibration verification percent recoveries must be within ± 30% of the spiked concentration.

Modify method to read:

14.5.1 At least one continuing calibration verification per ten samples must show a percent recovery within +/-30% of the spiked concentration.

Section 14.3.2

Method reads:

NA

Modify method to read:

14.3.2 The second (bracketing) calibration curve may be deactivated if instrumental drift affects the data. The first curve and acceptable calibration checks shall bracket usable data.

BACK TO MAIN

Study #: FACT-TOX-098

Section 14.3.3
Method reads:

NA

Modify method to read:

14.3.3 Calibration standards with peak areas less than 2 times the curve matrix blank should be deactivated to disqualify a data range that may be affected by background levels of the analyte.

Section 14.3.4
Method reads:

NA

Modify method to read:

14.3.4 Low or high curve points may be deactivated to optimize a linear range appropriate to the data.

Section 14.3.5
Method reads:

NA

Modify method to read:

14.3.5 A curve point may be deactivated if it deviates more than 30% from the theoretical value when the curve is evaluated over a linear range appropriate to the data.

Section 14.3.6
Method reads:

NA

Modify method to read:

14.3.6 A valid calibration curve must contain at least 5 active points.

Kam Hz 11/29/00
Signature of PAI and date

John Z. Butenhoff 13 DEC 2000
Signature of Sponsor and date

Warren T Case 13 Dec 2000
Signature of Study Director and date

Appendix D: Data Summary Tables

BACK TO MAIN

Table 7. Reported Fluorochemical Levels in Sera Analyses in Study FACT TOX-098

Dosage Group	Specimen ID	PFOS (µg/mL)	PFOSA (µg/mL)[a]	PFOSAA (µg/mL)[a]	EtFOSE-OH (µg/mL)[a]
Group 1 (Control) 0 mg/kg/day	12573F	<LOQ (0.0248)[a]	<LOQ (0.005)	<LOQ (0.0263)	<LOQ (0.0098)
	12574F	<LOQ (0.0248)[a]	<LOQ (0.005)	<LOQ (0.0263)	<LOQ (0.0098)
	12575F	<LOQ (0.0248)[a]	<LOQ (0.005)	<LOQ (0.0263)	<LOQ (0.0098)
Group 2 1 mg/kg/day	12576F	3.35[a]	0.0893	5.08	0.00706
	12577F	3.28[a]	0.0478	3.72	0.00397
	12578F	2.70[a]	0.0292*	2.18	0.00794
	12579F	3.69[a]	0.0786	6.01	<LOQ (0.00493)
	12580F	4.01[a]	0.0788	6.65	0.00848
Group 3 5 mg/kg/day	12581F	28.5[a]	0.500	56.1	0.0159
	12582F	24.7[a]	0.308*	48.0	0.0121
	12583F	16.2[a]	0.375*	14.4	0.0181
Group 4 10 mg/kg/day	12584F	32.0[a]	0.918	31.2	0.0406
	12585F	38.0[a]	0.888	39.1	0.0356
	12586F	34.5[a]	0.924	20.9	0.0352
Group 5 20 mg/kg/day	12587F	79.5[b]	1.72	48.3	0.0800
	12588F	73.1[b]	1.27	53.6	0.0661
	12589F	60.9[b]	1.27	33.7	0.0886
	12590F	75.9[b]	1.89	53.6	0.0762
	12591F	47.7[b]	1.08	26.5	0.105

LOQ—Limit of Quantitation
[a]Results have not been corrected for the purity of the analytical reference material.
[b]Results have been corrected for the purity of the analytical reference material.
*Tentative value, diluted extracts were below valid linear range of calibration curve.
It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data are quantitative to ±40%.

Table 8. Reported Fluorochemical Levels in Liver Analyses in Study FACT TOX-098

Dosage Group	Specimen ID	PFOS (µg/g)[a]	PFOSA (µg/g)[a]	PFOSAA (µg/g)[a]	EtFOSE-OH (µg/g)[a]
Group 1 (Control) 0 mg/kg/day	12573F	0.0994	<LOQ (0.120)	<LOQ (0.063)	<LOQ (0.0593)
	12574F	0.0253	<LOQ (0.120)	<LOQ (0.063)	<LOQ (0.0593)*
	12575F	0.137	0.148	<LOQ (0.063)	<LOQ (0.0593)*
Group 2 1 mg/kg/day	12576F	9.68	1.52	7.73	<LOQ (0.0593)*
	12577F	7.60	1.25	5.49	0.292*
	12578F	6.34	1.13	3.93	0.0774*
	12579F	8.58	1.39	8.81	0.166*
	12580F	7.00	1.24	7.95	0.132*
Group 3 5 mg/kg/day	12581F	38.9	5.65	58.6	1.56
	12582F	38.7	4.50	53.4	0.803
	12583F	33.1	3.54	21.3	0.854
Group 4 10 mg/kg/day	12584F	75.9	7.33	40.8	6.96
	12585F	85.8	6.07	58.4	5.26
	12586F	76.4	6.67	26.8	6.40
Group 5 20 mg/kg/day	12587F	145	9.18	96.1	15.3
	12588F	209	8.23	154	16.8
	12589F	167	6.51	76.1	16.5
	12590F	121	5.90	103	10.4
	12591F	114	6.96	97.0	12.5

LOQ—Limit of Quantitation
[a]Results have not been corrected for the purity of the analytical reference material.
*Data are qualitative only, matrix blank was unusually high and a suitable calibration curve (r^2 >0.98) could not be determined from this analysis.
It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data are quantitative to ±40%.

Table 9. Average Concentration of Fluorochemical Levels in Sera Analyses in Study FACT TOX-098

Dosage Group	PFOS (µg/mL)	PFOSA (µg/mL)[a]	PFOSAA (µg/mL)[a]	EtFOSE-OH (µg/mL)[a]
Group 1 (Control) 0.0 mg/kg/day	<LOQ[a]	<LOQ	<LOQ	<LOQ
Group 2 1 mg/kg/day	3.40[a]	0.0647	4.73	0.00686 1 Anomaly
Group 3 5 mg/kg/day	23.1[a]	0.395	39.5	0.0154
Group 4 10.0 mg/kg/day	34.8[a]	0.910	30.4	0.0371
Group 5 20.0 mg/kg/day	67.4[b]	1.44	43.1	0.0833

LOQ—Limit of Quantitation
[a] Results have not been corrected for the purity of the analytical reference material.
[b] Result has been corrected for purity of the analytical reference material.
It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data are quantitative to ±40%.

Table 10. Average Concentration of Fluorochemical Levels in Liver Analyses in Study FACT TOX-098

Dosage Group	PFOS (µg/g)[a]	PFOSA (µg/g)[a]	PFOSAA (µg/g)[a]	EtFOSE-OH (µg/g)[a]
Group 1 (Control) 0.0 mg/kg/day	0.0873	0.148	<LOQ	<LOQ
Group 2 1 mg/kg/day	7.84	1.31	6.78	0.167*
Group 3 5 mg/kg/day	36.9	4.56	44.4	1.07
Group 4 10.0 mg/kg/day	79.4	6.69	42.0	6.21
Group 5 20.0 mg/kg/day	151	7.36	105	14.3

LOQ—Limit of Quantitation
[a] Results have not been corrected for the purity of the analytical reference material.
* Qualitative only
It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data are quantitative to ±40%.

Appendix E: Data Spreadsheets

FACT-TOX-098
Argus# 418-011

Study: Argus 418-011, Oral Developmental Toxicity Study of BtFOSE-OH in Rats
Product Number(Test Substance): BtFOSE-OH (T-6316.7)
Matrix: Rat Serum
Method/Revision: FACT-M-3.1 & FACT-M-4.1 - linear regression, weighted 1/x
Analytical Equipment System Number: Madeline 041098
Instrument Software/Version: MassLynx 3.1
Filename: See listing to the right
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments
Dates of Extraction/Analyst: 9/30/98 SAH/JCP
Dates of Analysis/Analyst: 10/01/98, 10/05/98, 09/29/99 HOJ/KJH/LAS
Date of Data Reduction/Analyst: 10/18/00 KJH

On 10/05/98
files from 050-110 dilutions included on the summary

On 09/29/99
files from 1-95 dilutions not included on the summary

TOX-012 Curve RTS091698
10/05/98 PFOS, Lot 193
10/05/98 PFOSA, Lot L-2353
10/05/98 PFOSAA, Lot 617
10/05/98 PFOSEA, Lot TN-A-1885
10/05/98 BtFOSE, Lot 936
10/05/98 M556, Calc. using PFOSAA Curve

TOX-029 Curve RBS032599
9/29/99 PFOS SD009, Lot 171
9/29/99 PFOSA SD010, Lot L-2353
NB 112999-99 9/29/99 PFOSAA SD006, Lot T-7121.1
9/29/99 PFOSEA SD012, Lot 529
9/29/99 BtFOSE SD013, Lot 936
9/29/99 M556 SD014, Lot NB# 113047-80

Filenames
PFOS Finals
Blks M100598051-52 & 101-102
Grp 1 100598062-64
Grp 2 100598068-72
Grp 3 100598076-78
Grp 4 100598082-84
Grp 5 092999035-42

MS, MSD 092999054-58

Sample Data
RAT SERA F0

lot 193, 171 (PFOS) — lot L-2353 (PFOSA)

Group Dose	Sample #	Extraction Vol. Ratio	PFOS Std Correction Factor	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec.	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD	PFOSA Purity Correction Factor	PFOSA Dilution Factor	PFOSA Conc. ng/mL	Concentration of PFOSA ug/mL or % Rec.	Mean PFOSA ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	1	0.9275	Unknown	1	0.00	<LOQ (24.8 ng/mL)			Unknown	1	0.00	<LOQ (5.00 ng/mL)		
	H2O Blk-2	1	0.9275	Unknown	1	0.00	<LOQ (24.8 ng/mL)	<LOQ	NA	Unknown	1	0.00	<LOQ (5.00 ng/mL)	<LOQ	NA
Matrix Blk	Rat Serum Blk-1	1	0.9275	Unknown	1	0.00	<LOQ (24.8 ng/mL)			Unknown	1	0.00	<LOQ (5.00 ng/mL)		
	Rat Serum Blk-2	1	0.9275	Unknown	1	0.00	<LOQ (24.8 ng/mL)	<LOQ	NA	Unknown	1	0.00	<LOQ (5.00 ng/mL)	<LOQ	NA
QC-100 ppb	12574F-MS	1	NA	NA	1	118	121%			NA	1	102	103%		
	12574F-MSD	1	NA	NA	1	149	152%	137%	23%	NA	1	147	149%	126%	37%
Group 1	12573F	1	0.9275	Unknown	1	17.8	<LOQ (24.8 ng/mL)			Unknown	1	0.00	<LOQ (5.00 ng/mL)		
Control 0.0 mg/mL	12574F	1	0.9275	Unknown	1	0.00	<LOQ (24.8 ng/mL)			Unknown	1	0.00	<LOQ (5.00 ng/mL)		
0.0 mg/kg/day	12575F	1	0.9275	Unknown	1	19.4	<LOQ (24.8 ng/mL)	<LOQ	MNA	Unknown	1	0.00	<LOQ (5.00 ng/mL)	<LOQ	NA
Group 2	12576F	1	0.9275	Unknown	10	363	3.35			Unknown	10	8.97	0.0893		
1 mg/kg/day	12577F	1	0.9275	Unknown	10	355	3.28			Unknown	10	4.80	0.0478		
0.2 mg/mL	12578F	1	0.9275	Unknown	10	292	2.70			Unknown	10	2.93	0.0292 *		
	12579F	1	0.9275	Unknown	10	400	3.69		14.5	Unknown	10	7.90	0.0786		39.0
	12580F	1	0.9275	Unknown	10	434	4.01	3.40	0.492	Unknown	10	7.92	0.0788	0.0647	0.0252
Group 3	12581F	1	0.9275	Unknown	100	308	28.5			Unknown	100	5.03	0.500		
5 mg/kg/day	12582F	1	0.9275	Unknown	100	267	24.7		27.3	Unknown	100	3.10	0.308 *		24.7
1.0 mg/mL	12583F	1	0.9275	Unknown	100	175	16.2	23.1	6.30	Unknown	100	3.77	0.375 *	0.395	0.0975
Group 4	12584F	1	0.9275	Unknown	100	347	32.0			Unknown	100	9.23	0.918		
10.0 mg/kg/day	12585F	1	0.9275	Unknown	100	412	38.0		8.68	Unknown	100	8.92	0.888		2.17
2.0 mg/mL	12586F	1	0.9275	Unknown	100	374	34.5	34.8	3.02	Unknown	100	9.29	0.924	0.910	0.0198
Group 5	12587F	1	0.9275	0.8640	2000	49.8	79.5			Unknown	100	17.3	1.72		
20.0 mg/kg/day	12588F	1	0.9275	0.8640	2000	45.8	73.1			Unknown	100	12.7	1.27		
4.0 mg/mL	12589F	1	0.9275	0.8640	2000	38.2	60.9			Unknown	100	12.7	1.27		
	12590F	1	0.9275	0.8640	2000	47.6	75.9		19.3	Unknown	100	19.0	1.89		23.9
	12591F	1	0.9275	0.8640	2000	29.9	47.7	67.4	13.0	Unknown	100	10.8	1.08	1.44	0.345

Correction factors not applicable for MS/MSD QC data

PFOS = Perfluorooctanesulfonate
* Tentative value, below range of calibration curve. LAC 10/31/00

Date Entered/By: 10/29/00 LAC
Date Verified/ By: 10/31/00 MMH
Extraction Volume Ratio = Initial volume/final volume. For all samples and standards the initial volume is equal to the final volume for an extraction volume ratio of 1.

FACT-TOX-098
Argus# 418-011

Study:	Argus 418-011, Oral Developmental Toxicity Study of EtFOSE-OH in Rats
Product Number(Test Substance):	EtFOSE-OH (T-6316.7)
Matrix:	Rat Serum
Method/Revision:	FACT-M-3.1 & FACT-M-4.1 - linear regression, weighted 1/x
Analytical Equipment System Number:	Madeline 041098
Instrument Software/Version:	MassLynx 3.1
Filename:	See listing to the right
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	9/30/98 SAH/JCP
Dates of Analysis/Analyst:	10/01/98, 10/05/98, 09/29/99 ROJ/KJH/IAS
Date of Data Reduction/Analyst:	10/18/00 KJH

Filenames

	PFOSA Female	PFOSAA Female	EtFOSE Female	M556 Female	Female Dil.
Blks	M100598051-52 & 101-102	M100598051-52 & 101-102	M100598051-52 & 101-102	S092999004,6 & 100598	1/1, 1/1, 1/1, 1/1, 1/1
Grp 1	100598062-64	100598062-64	100598062-64	092999051-53	1/1, 1/1, 1/1, 1/1, 1/1
Grp 2	100598068-72	100598068-72	092999059-66	092999018-22	1/10, 1/10, 1/10, 1/1, 1/100
Grp 3	100598076-78	100598076-78	092999067-69	092999026-28	1/100, 1/100, 1/100, 1/1, 1/1000
Grp 4	100598082-84	100598082-84	092999070-75	092999029-34	1/100, 1/100, 1/100, 1/1, 1/1000
Grp 5	100598088-92	100598088-92	092999076-83	092999043-50	1/2000, 1/100, 1/100, 1/100, 1/200
MS, MSD	092999054-58	092999054-58	092999054-58	092999054-58	1/1, 1/1, 1/1, 1/1

Sample Data

RAT SERA F0

lot 617, NB 112999-99 lot 936 lot using P

Group Dose	Sample #	PFOSAA Purity Correction Factor	PFOSAA Dilution Factor	PFOSAA Conc. ng/mL	Concentration of PFOSAA ug/mL or % Rec.	Mean PFOSAA ug/mL	RSD Std. Dev. MS/MSD RPD	EtFOSE Purity Correction Factor	EtFOSE Dilution Factor	EtFOSE Conc. ng/mL	Concentration of EtFOSE ug/mL or % Rec.	Mean EtFOSE ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	Unknown	1	0.00	<LOQ (26.3 ng/mL)			Unknown	1	0.00	<LOQ (9.80 ng/mL)		
	H2O Blk-2	Unknown	1	0.00	<LOQ (26.3 ng/mL)	<LOQ	NA	Unknown	1	0.00	<LOQ (9.80 ng/mL)	<LOQ	NA
Matrix Blk	Rat Serum Blk-1	Unknown	1	0.00	<LOQ (26.3 ng/mL)			Unknown	1	0.00	<LOQ (9.80 ng/mL)		
	Rat Serum Blk-2	Unknown	1	0.00	<LOQ (26.3 ng/mL)	<LOQ	NA	Unknown	1	0.00	<LOQ (9.80 ng/mL)	<LOQ	NA
QC-100 ppb	12574F-MS	NA	1	80.6	78%			NA	1	78.7	80%		
	12574F-MSD	NA	1	94.3	91%	84%	16%	NA	1	96.0	98%	89%	20%
Group 1	12573F	Unknown	1	2.92	<LOQ (26.3 ng/mL)			Unknown	1	0.00	<LOQ (9.80 ng/mL)		
Control 0.0 mg/mL	12574F	Unknown	1	0.00	<LOQ (26.3 ng/mL)			Unknown	1	0.00	<LOQ (9.80 ng/mL)		
0.0 mg/kg/day	12575F	Unknown	1	6.93	<LOQ (26.3 ng/mL)	<LOQ	NA	Unknown	1	0.00	<LOQ (9.80 ng/mL)	<LOQ	NA
Group 2	12576F	Unknown	10	510	5.08			Unknown	1	7.10	0.00706		
1 mg/kg/day	12577F	Unknown	10	374	3.72			Unknown	1	3.99	0.00397		
0.2 mg/mL	12578F	Unknown	10	219	2.18			Unknown	1	7.98	0.00794		
	12579F	Unknown	10	604	6.01		38.0	Unknown	1	0.00	<LOQ (4.93 ng/mL)	0.00686	29.4
	12580F	Unknown	10	668	6.65	4.73	1.80	Unknown	1	8.52	0.00848	0.00686 - 1 Anomaly	0.00201
Group 3	12581F	Unknown	100	564	56.1			Unknown	1	16.0	0.0159		
5 mg/kg/day	12582F	Unknown	100	482	48.0		56.0	Unknown	1	12.2	0.0121		19.6
1.0 mg/mL	12583F	Unknown	100	145	14.4	39.5	22.1	Unknown	1	18.2	0.0181	0.0154	0.00301
Group 4	12584F	Unknown	100	314	31.2			Unknown	1	40.8	0.0406		
10.0 mg/kg/day	12585F	Unknown	100	393	39.1		30.0	Unknown	1	35.8	0.0356		8.15
2.0 mg/mL	12586F	Unknown	100	210	20.9	30.4	9.12	Unknown	1	35.4	0.0352	0.0371	0.00303
Group 5	12587F	Unknown	100	485	48.3			Unknown	1	80.4	0.0800		
20.0 mg/kg/day	12588F	Unknown	100	539	53.6			Unknown	1	66.5	0.0661		
4.0 mg/mL	12589F	Unknown	100	339	33.7			Unknown	1	89.0	0.0886		
	12590F	Unknown	100	539	53.6		28.7	Unknown	1	76.6	0.0762		17.7
	12591F	Unknown	100	266	26.5	43.1	12.4	Unknown	1	106	0.105	0.0833	0.0147

Correction factors not applicable for MS/MSD QC data

PFOS = Perfluorooctanesulfonate

* Tentative value, below range of calibration curve. LAC 10/31/00

Date Entered/By: 10/29/00 LAC
Data Verified/ By: 10/31/00 MMH
Extraction Volume Ratio = Initial volume/final volume. For all samples and standards the initial volume is equal to the final volume for an extraction volume ratio of 1.

FACT-TOX-098
Argus# 418-011

Study:	Argus 418-011. Oral Developmental Toxicity Study of EtFOSE-OH in Rats
Product Number(Test Substance):	EtFOSE-OH (T-6316.7)
Matrix:	Rat Liver
Method/Revision:	FACT-M-1.0 & ETS-8-5.1, linear regression, weighted 1/x
Analytical Equipment System Number:	Amelia 062498, Madeline 041098, Soup 020199
Instrument Software/Version:	MassLynx 3.1, 3.3, 3.4
Date of Extraction/Analyst:	10/2/98 JCP/ IAS
Date of Analysis/Analyst:	10/02/98, 10/06/98, 09/28/99 MEE/HOJ/IAS
Date of Data Reduction/Analyst:	09/29/99, 10/17/00, 10/18/00 IAS/KJH

Filename: See list to right
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments

On 10/06/98
files from 56-124 dilution included in summary
files from 115-184 dilution not included in summary
All other dates, dilutions not included in summary

10/02/98 Analysis:
May need to calc. Against PFOSAA
curve files 10/06/98 analysis
Calculated using PFOSAA Curve

Filenames	PFOS Female	PFOSA Female	PFOSAA Female	Qualitative Data Only EtFOSE Female	Qualitative Data Only PFOSEA Female	M556 Female	Dilutions
Blks	A100698057-58 & 115-116	A100298003-4 & 61-62	A100298003-4 & 61-62	A100298003-4 & 61-62	A100298003-4 & 61-62	A100698057-58 & 115-116	
Grp 1	092899044, 100298016-17	100298015-17	100298015-17	092899044, 100298016-17	100298015-17	10/02/98 Not analyzed for M556	1/1, 1/1, 1/1, 1/1, 1/1
Grp 2	100698075-79	100698082-86	100698075-79	100298021-25	100298021-25	100698082-86	1/20, 1/10, 1/20, 1/1, 1/10
Grp 3	100698094-96	100696099-101	100698094-96	100698104-106	100298031-33	100698094-96	1/100, 1/20, 1/100, 1/2, 1/100
Grp 4	100698109-111	100698132-134	100698127-129	100698132-134	100298039-41	100698127-129	1/500, 1/20, 1/100, 1/20, 1/100
Grp 5	100698142-146	100698156-160	100698142-146	100698149-153	100298047-51	100698142-146	1/1000, 1/20, 1/1000, 1/100, 1/1000
MS, MSD	092899045-46	100298057-58	100298057-58	092899045-46	100298057-58	NS	1/1, 1/1, 1/1, 1/1, 1/1

Sample Data

RAT LIVER F0

lot 193 lot L-2353

Group - Dose	Sample #	Initial Wt. g	Total Mass of Liver g	PFOS Std Correction Factor	PFOS Purity Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	PFOSA Purity Correction Factor	PFOSA Conc. ng/g	PFOSA Dilution Factor	PFOSA Calc. Conc. ng/g	Concentration of PFOSA ug/g or % Rec.	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	1.0000	NA	0.9275	Unknown	23.4	1	21.7	<LOQ (59.2 ng/g)			Unknown	13.7	1	13.7	<LOQ (120 ng/g)		
	H2O Blk-2	1.0000	NA	0.9275	Unknown	25.1	1	23.2	<LOQ (59.2 ng/g)	<LOQ	NA	Unknown	20.0	1	20.0	<LOQ (120 ng/g)	<LOQ	NA
Matrix Blk	Rabbit Liver Blk-1	1.0000	40.13	0.9275	Unknown	24.9	1	23.1	<LOQ (59.2 ng/g)			Unknown	24.5	1	24.5	<LOQ (120 ng/g)		N
	Rabbit Liver Blk-2	1.0000	40.13	0.9275	Unknown	25.1	1	23.3	<LOQ (59.2 ng/g)	<LOQ	NA	Unknown	25.7	1	25.7	<LOQ (120 ng/g)	<LOQ	NA
QC - 100 ppb	12573F-MS	1.0118	NA	NA	NA	237	1	136	115%			NA	174	1	155	129%		
	12573F-MSD	1.0118	NA	NA	NA	217	1	117	99%	107%	15%	NA	159	1	139	116%	122%	10%
Group 1	12573F	1.0118	NA	0.9275	Unknown	108	1	99.4	0.0994			Unknown	17.9	1	17.7	<LOQ (120 ng/g)		
Control 0.0 mg/mL	12574F	1.0111	NA	0.9275	Unknown	27.6	1	25.3	0.0253		65.2	Unknown	27.6	1	27.3	<LOQ (120 ng/g)		NA
0.0 mg/kg/day	12575F	1.0116	NA	0.9275	Unknown	150	1	137	0.137	0.0873	0.0570	Unknown	150	1	148	0.148	0.148	NA
Group 2	12576F	1.0101	NA	0.9275	Unknown	527	20	9680	9.68			Unknown	154	10	1521	1.52		
1 mg/kg/day	12577F	1.0122	NA	0.9275	Unknown	415	20	7603	7.60			Unknown	127	10	1252	1.25		
0.2 mg/mL	12578F	0.9973	NA	0.9275	Unknown	341	20	6342	6.34			Unknown	113	10	1134	1.13		
	12579F	0.9970	NA	0.9275	Unknown	461	20	8583	8.58		16.8	Unknown	139	10	1389	1.39		11.5
	12580F	1.0015	NA	0.9275	Unknown	578	20	6999	7.00	7.84	1.32	Unknown	124	10	1237	1.24	1.31	0.151
Group 3	12581F	1.0063	NA	0.9275	Unknown	422	100	38929	38.9			Unknown	284	20	5645	5.65		
5 mg/kg/day	12582F	1.0200	NA	0.9275	Unknown	426	100	38747	38.7		8.96	Unknown	229	20	4497	4.50		23.1
1.0 mg/mL	12583F	1.0066	NA	0.9275	Unknown	359	100	33110	33.1	36.9	3.31	Unknown	178	20	3539	3.54	4.56	1.05
Group 4	12584F	1.0149	NA	0.9275	Unknown	166	500	75889	75.9			Unknown	372	20	7331	7.33		
10.0 mg/kg/day	12585F	1.0129	NA	0.9275	Unknown	187	500	85809	85.8		7.04	Unknown	307	20	6071	6.07		9.41
2.0 mg/mL	12586F	1.0098	NA	0.9275	Unknown	166	500	76382	76.4	79.4	5.59	Unknown	337	20	6674	6.67	6.69	0.630
Group 5	12587F	1.0116	NA	0.9275	Unknown	158	1000	144801	145			Unknown	464	20	9179	9.18		
20.0 mg/kg/day	12588F	1.0200	NA	0.9275	Unknown	250	1000	208742	209			Unknown	420	20	8230	8.23		
4.0 mg/mL	12589F	1.0063	NA	0.9275	Unknown	181	1000	166624	167			Unknown	328	20	6514	6.51		
	12590F	1.0044	NA	0.9275	Unknown	131	1000	121367	121		25.3	Unknown	296	20	5900	5.90		18.1
	12591F	1.0072	NA	0.9275	Unknown	124	1000	114249	114	151	38.2	Unknown	351	20	6962	6.96	7.36	1.35

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
PFOSEA = Perfluorooctane sulfonyl ethylamide
M556 = Perfluorooctanesulfonylamidoacetate $C_8F_{17}SO_2N(H)CH_2COO)$
Correction factors not applicable to MS/MSD QC data

10/02/98 analysis - PFOSAA curve included four points instead of five. LAC 10/18/00
**EtFOSE and PFOSEA data from 10/02/98 are qualitative only: a suitable calibration curve could not be achieved. LAC 10/30/00
NS = Not spiked

Data Entered/Analyst: 10/02/98, 10/18/00, 10/30/00 LAC
Data Verified/Analyst: 10/31/00 MMH

FACT-TOX-098
Argus# 418-011

Study: Argus 418-011, Oral Developmental Toxicity Study of EtFOSE-OH in Rats
Product Number(Test Substance): EtFOSE-OH (T-6316.7)
Matrix: Rat Liver
Method/Revision: FACT-M-1.0 & ETS-8-5.1, linear regression, weighted 1/x
Analytical Equipment System Number: Amelia 062498, Madeline 041098, Soup 020199
Instrument Software/Version: MassLynx 3.1, 3.3, 3.4
Date of Extraction/Analyst: 10/2/98 JCW IAS
Date of Analysis/Analyst: 10/02/98, 10/06/98, 09/28/99 MEB/HO/IAS
Date of Data Reduction/Analyst: 09/29/99, 10/17/00, 10/18/00 IAS/KJH

Filename: See list to right
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments

Filenames	PFOS Female	PFOSA Female	PFOSAA Female	EtFOSE Female	PFOSEA Female	M556 Female	Dilutions
Blks	A100698057-58 & 115-116	A100298003-4 & 61-6[illegible]	A100298003-4 & 61-62	A100298003-4 & 61-62	A100298003-4 & 61-62	A100698057-58 & 115-116	0
Grp 1	092899044, 100298016-17	100298015-17	100298015-17	092899044, 100298016-17	100298015-17	10/02/98 Not analyzed for M556	1/1, 1/1, 1/1, 1/1, 1/1
Grp 2	100698075-79	100698082-86	100698075-79	100298021-25	100298021-25	100698082-86	1/20, 1/10, 1/20, 1/1, 1/10
Grp 3	100698094-96	100696099-101	100698094-96	100698104-106	100298031-33	100698094-96	1/100, 1/20, 1/100, 1/2, 1/100
Grp 4	100698109-111	100698132-134	100698127-129	100698132-134	100298039-41	100698127-129	1/500, 1/20, 1/100, 1/20, 1/100
Grp 5	100698142-146	100698156-160	100698142-146	100698149-153	100298047-51	100698142-146	1/1000, 1/20, 1/1000, 1/100, 1/1000
MS, MSD	092899045-46	100298057-58	100298057-58	092899045-46	100298057-58	NS	1/1, 1/1, 1/1, 1/1, 1/1

Sample Data

RAT LIVER F0

lot 617 (PFOSAA); lot 936 (EtFOSE)

Group Dose	Sample #	PFOSAA Purity Correction Factor	PFOSAA Conc. ng/g	PFOSAA Dilution Factor	PFOSAA Calc. Conc. ng/g	Concentration of PFOSAA ug/g or % Rec.	Mean PFOSAA ug/g	RSD Std. Dev. MS/MSD RPD	EtFOSE Purity Correction Factor	EtFOSE Conc. ng/g	EtFOSE Dilution Factor	EtFOSE Calc. Conc. ng/g	Concentration of EtFOSE ug/g or % Rec.		Mean EtFOSE ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	Unknown	0.00	1	0.00	<LOQ (63 ng/g)			Unknown	828	1	828	0.828	**		
	H2O Blk-2	Unknown	0.00	1	0.00	<LOQ (63 ng/g)	<LOQ	NA	Unknown	354	1	354	0.354	**	0.591	0.336
Matrix Blk	Rabbit Liver Blk-1	Unknown	0.00	1	0.00	<LOQ (63 ng/g)			Unknown	0.00	1	0.00	<LOQ (59.3 ng/g)	**		
	Rabbit Liver Blk-2	Unknown	0.00	1	0.00	<LOQ (63 ng/g)	<LOQ	NA	Unknown	0.00	1	0.00	<LOQ (59.3 ng/g)	**	<LOQ	NA
QC - 100 ppb	12573F-MS	NA	125	1	124	98%			NA	109	1	108	91%			
	12573F-MSD	NA	132	1	131	104%	101%	5%	NA	105	1	104	87%		89%	4%
Group 1 Control 0.0 mg/mL 0.0 mg/kg/day	12573F	Unknown	55.3	1	54.6	<LOQ (63 ng/g)			Unknown	0.00	1	0.00	<LOQ (59.3 ng/g)			
	12574F	Unknown	0.00	1	0.00	<LOQ (63 ng/g)		NA	Unknown	34.1	1	33.7	<LOQ (59.3 ng/g)	**		NA
	12575F	Unknown	0.00	1	0.00	<LOQ (63 ng/g)	<LOQ	NA	Unknown	44.2	1	43.7	<LOQ (59.3 ng/g)	**	<LOQ	NA
Group 2 1 mg/kg/day 0.2 mg/mL	12576F	Unknown	390	20	7727	7.73			Unknown	26.7	1	26.4	<LOQ (59.3 ng/g)	**		
	12577F	Unknown	278	20	5487	5.49			Unknown	295	1	292	0.292	**		
	12578F	Unknown	196	20	3934	3.93			Unknown	77.2	1	77.4	0.0774	**		
	12579F	Unknown	439	20	8809	8.81		29.6	Unknown	166	1	166	0.166	**		54.5
	12580F	Unknown	398	20	7952	7.95	6.78	2.01	Unknown	132	1	132	0.132	**	0.167	0.0910
Group 3 5 mg/kg/day 1.0 mg/mL	12581F	Unknown	590	100	58615	58.6			Unknown	785	2	1560	1.56			
	12582F	Unknown	545	100	53408	53.4		45.5	Unknown	410	2	803	0.803			39.4
	12583F	Unknown	214	100	21296	21.3	44.4	20.2	Unknown	430	2	854	0.854		1.07	0.423
Group 4 10.0 mg/kg/day 2.0 mg/mL	12584F	Unknown	414	100	40824	40.8			Unknown	353	20	6960	6.96			
	12585F	Unknown	592	100	58426	58.4		37.7	Unknown	266	20	5260	5.26			14.0
	12586F	Unknown	271	100	26787	26.8	42.0	15.9	Unknown	323	20	6402	6.40		6.21	0.867
Group 5 20.0 mg/kg/day 4.0 mg/mL	12587F	Unknown	97.2	1000	96076	96.1			Unknown	155	100	15298	15.3			
	12588F	Unknown	157	1000	153608	154			Unknown	171	100	16775	16.8			
	12589F	Unknown	76.6	1000	76120	76.1			Unknown	166	100	16484	16.5			
	12590F	Unknown	104	1000	103286	103		27.5	Unknown	105	100	10437	10.4			19.1
	12591F	Unknown	97.8	1000	97023	97.0	105	28.9	Unknown	126	100	12524	12.5		14.3	2.74

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
PFOSEA = Perfluorooctane sulfonyl ethylamide
M556 = Perfluorooctanesulfonylamidoacetate C8F17SO2N((H)CH2COO)
Correction factors not applicable to MS/MSD QC data

10/02/98 analysis - PFOSAA curve included four points instead of five. LAC 10/18/00
**EtFOSE and PFOSEA data from 10/02/98 are qualitative only; a suitable calibration curve could not be achieved. LAC 10/30/00
NS = Not spiked

Date Entered/Analyst: 10/02/98, 10/18/00, 10/30/00 LAC
Date Verified/Analyst: 10/31/00 MMH

Appendix F: Example Calculations

Formula Used for Sera Analyses in Study FACT TOX-098

$$AR\ (ng/mL) \times DF \times SC \times \frac{FV\ (mL)}{EV\ (mL)} \times \frac{1.0\ \mu g}{1000\ ng} = \textbf{Reported Concentration } (\mu g/mL)$$

Calculation Used for Group 2, Animal ID 12576F

$$363\ ng/mL \times 10 \times 0.9275 \times \frac{1\ mL}{1.005\ mL} \times \frac{1.0\ \mu g}{1000\ ng} = \mathbf{3.35\ \mu g/mL}$$

AR— Analytical result from MassLynx summary
DF— Dilution factor
SC—PFOS salt correction constant (0.9275)
FV—Final extract volume (1.0 mL unless otherwise noted)
EV—Volume of sera extracted

Formula Used for Liver Analyses in Study FACT TOX-098

$$AR\ (ng/g) \times \frac{\partial\ curve^{(1)}}{\partial\ sample} \times SC \times DF \times \frac{1.0\ \mu g}{1000\ ng} = \textbf{Reported Concentration } (\mu g/g)$$

(1) ∂ curve is assumed to be: $\frac{1\ g\ liver}{5\ mL\ H_2O}$

Calculation Used for Group 2, Animal ID 12576F

$$527\ ng/g \times \frac{1\ g/\ 5\ mL}{1.0101\ g/\ 5mL} \times 0.9275 \times 20 \times \frac{1.0\ \mu g}{1000\ ng} = \mathbf{9.68\ \mu g/g}$$

AR— Analytical result from MassLynx summary
∂ curve—Density of the liver standard curve, assumed to be 1g liver/ 5 ml water
∂ sample—Density of the liver sample (g sample/ 5 mL H_2O)
SC—PFOS salt correction constant (0.9275)
DF— Dilution factor

Appendix G: Interim Certificates of Analysis

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

Centre Analytical Laboratories COA Reference #: 023-018B
3M Product: PFOS, Lot 171
Reference #: SD-009
Purity: 86.4%

Test Name	Specifications	Result
Purity[1]		**86.4%**
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		1. 0.017 wt./wt.% 2. 0.007 wt./wt.% 3. 1.355 wt./wt.% 4. 6.552 wt./wt.% 5. 0.003 wt./wt.% 6. 0.004 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.00 wt./wt.%
Total % Impurity (LC/MS)		10.60 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		0.30 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable[3]
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate[4]		1. <0.015 wt./wt.% 2. 0.27 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 8.82 wt./wt.%
Organic Acids [5] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. <0.1 wt./wt.% 4. <0.25 wt./wt.%
Elemental Analysis[6]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	1. Theoretical Value = 17.8% 2. Theoretical Value = 0% 3. Theoretical Value = 0% 4. Theoretical Value = 5.95% 5. Theoretical Value = 60%	1. 12.08 wt./wt.% 2. 0.794 wt./wt.% 3. 1.61 wt./wt.% 4. 10.1 wt./wt.% 5. 50.4 wt./wt.%

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018B

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

[1]Purity = 100% - (sum of metal impurities, 1.39% +LC/MS impurities, 10.60%+Inorganic Fluoride, 0.27%+NMR impurities, 1.00%+ POAA, 0.30%)

Total impurity from all tests = 13.56%
Purity = 100% - 13.56% = 86.4%

[2]Potassium is expected in this salt form and is therefore not considered an impurity.

[3]Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

[4]Sulfur in the sample appears to be converted to SO_4 and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the SO_4 is not considered an impurity.

[5]TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonofluoropentanoic acid
PFPA	Pentafluoropropanoic acid

[6]Theoretical value calculations based on the empirical formula, $C_8F_{17}SO_3^-K^+$ (MW=538)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018B

LC/MS Purity Profile:

Impurity	wt./wt. %
C4	1.03
C5	1.56
C6	6.38
C7	1.63
Total	**10.60**

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average response factors from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average response factors from the C6 and C8 standard curves.

Prepared By: David S. Bell — 9/10/00
David S. Bell
Scientist, Centre Analytical Laboratories
Date

Reviewed By: John M Flaherty — 9/11/00
John Flaherty
Laboratory Manager, Centre Analytical Laboratories
Date

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-1
3M Product: EtFOSE-OH
Test Control Reference #: SD-013
Purity: 88.9%

Test Name	Specifications	Result
Purity[1]		**88.9%**
Appearance	Yellow-white, waxy solid	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium 5. Nickel 6. Iron 7. Manganese		1. <0.001 wt./wt.% 2. <0.001 wt./wt.% 3. <0.001 wt./wt.% 4. 0.002 wt./wt.% 5. <0.001 wt./wt.% 6. <0.001 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		0.90 wt./wt.%
Total % Impurity (LC/MS)		None Quantified
Total % Impurity (GC/MS)		10.21 wt./wt.%
Related Compounds – POAA		0.03 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		87.6 wt./wt.%.
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate		1. <0.015 wt./wt.% 2. <0.005 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. <0.040 wt./wt.%
Organic Acids[2] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. <0.1 wt./wt.% 4. <0.25 wt./wt.%
Elemental Analysis[3]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	1. Theoretical Value = 25.2% 2. Theoretical Value = 1.75% 3. Theoretical Value = 2.45% 4. Theoretical Value = 5.60% 5. Theoretical Value = 56.6%	1. 24.42 wt./wt.% 2. 1.78 wt./wt.% 3. 2.72 wt./wt.% 4. 9.34 wt./wt.% 5. 58.4 wt./wt.%

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-1
3M Product: EtFOSE-OH
Test Control Reference #: SD-013

Date of Last Analysis: 11/26/00

Expiration Date: 11/26/01

Storage Conditions: <-10 °C

Re-assessment Date: 11/26/01

[1]Purity = 100% - (total metal impurities, 0.002% + total NMR impurities, 0.90% + GC/MS impurities, 10.21 + POAA, 0.03%)

Total impurity from all tests = 11.14%
Purity = 100% - 11.14% = 88.9%

[2]TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonafluoropentanoic acid
PFPA	Pentafluoropropanoic acid

[3]Theoretical value calculations based on the empirical formula, $C_{12}H_{10}F_{17}NO_3S$ (MW=571)

Centre Analytical Laboratories, Inc.
3048 Research Drive
State College, PA 16801
Phone: (814) 231-8032
Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-1
3M Product: EtFOSE-OH
Test Control Reference #: SD-013

GC/MS Purity Profile

Peak #	Retention Time (min)	Identity	% Impurity
1	6.163	Unknown	0.12
2	8.011	Unknown	0.23
3	8.206	Unknown	0.51
4	9.065	Unknown	0.21
5	9.844	Unknown	0.34
6	13.93	Unknown	0.62
7	14.238	Unknown	0.11
8	15.130	C2	0.11
9	15.52	C3	1.11
10	15.941	C4	1.55
11	16.379	C5	1.07
12	16.801	C6	3.30
13	17.222	C7	0.93
Total	-	-	**10.21**

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

Prepared By: David S. Bell — 11/27/00
David S. Bell
Scientist
Centre Analytical Laboratories
Date

Reviewed By: John M Flaherty — 11/27/00
John Flaherty
Laboratory Manager
Centre Analytical Laboratories
Date

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-2
3M Product: EtFOSE-OH
Test Control Reference #: TCR-00017-52
Purity: 97.4%

Test Name	Specifications	Result
Purity[1]		**97.4%**
Appearance	Yellow-white, waxy solid	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium 5. Nickel 6. Iron 7. Manganese		1. <0.001 wt./wt.% 2. <0.001 wt./wt.% 3. <0.001 wt./wt.% 4. <0.001 wt./wt.% 5. <0.001 wt./wt.% 6. <0.001 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.26 wt./wt.%
Total % Impurity (LC/MS)		None Quantified
Total % Impurity (GC/MS)		1.29 wt./wt.%
Related Compounds – POAA		0.10 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		90.3 wt./wt.%.
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate		1. <0.015 wt./wt.% 2. <0.005 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. <0.154 wt./wt.%
Organic Acids[2] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. <0.1 wt./wt.% 4. <0.25 wt./wt.%
Elemental Analysis[3]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	 1. Theoretical Value = 25.2% 2. Theoretical Value = 1.75% 3. Theoretical Value = 2.45% 4. Theoretical Value = 5.60% 5. Theoretical Value = 56.6%	 1. 25.04 wt./wt.% 2. 1.69 wt./wt.% 3. 2.61 wt./wt.% 4. 8.88 wt./wt.% 5. 56.8 wt./wt.%

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-2
3M Product: EtFOSE-OH
Test Control Reference #: TCR-00017-52

Date of Last Analysis: 11/26/00

Expiration Date: 11/26/01

Storage Conditions: <-10 °C

Re-assessment Date: 11/26/01

[1]Purity = 100% - (total NMR impurities, 1.26% + GC/MS impurities, 1.29 + POAA, 0.10%)

Total impurity from all tests = 2.65%
Purity = 100% - 2.65% = 97.4%

[2]TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonafluoropentanoic acid
PFPA	Pentafluoropropanoic acid

[3]Theoretical value calculations based on the empirical formula, $C_{12}H_{10}F_{17}NO_3S$ (MW=571)

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-2
3M Product: EtFOSE-OH
Test Control Reference #: TCR-00017-52

GC/MS Purity Profile

Peak #	Retention Time (min)	Identity	% Impurity
1	13.934	PFOSDEA	0.36
2	17.307	C7	0.93
Total	-	-	**1.29**

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

Prepared By: David S. Bell — Date: 11/27/00
David S. Bell
Scientist
Centre Analytical Laboratories

Reviewed By: John M Flaherty — Date: 11/27/00
John Flaherty
Laboratory Manager
Centre Analytical Laboratories

BACK TO MAIN

Appendix H: Report Signature Page

Marvin T Case — 6 February 2001

Marvin T. Case, D.V.M., Ph.D., *Study Director* — Date

John L. Butenhoff — 6 FEB 2001

John L. Butenhoff, Ph.D., *Sponsor Representative* — Date

02/02/01

Kristen J. Hansen, Ph.D., *Principal Analytical Investigator* — Date

02/02/01

William K. Reagen, *Laboratory Manager* — Date